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Nemoto et al.

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(54) **APPARATUS FOR AUTOMATED PREPARATION OF DNA SAMPLES AND REACTOR FOR PREPARING DNA SAMPLES**

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Mar. 30, 2001 (JP) 2001-099080

(51) **Int. Cl.**[7] **C12M 1/34**
(52) **U.S. Cl.** **435/287.2**; 435/286.2; 435/286.4; 435/287.3; 435/288.7; 422/82.08; 422/100
(58) **Field of Search** 435/286.2, 286.4, 435/287.2, 287.3, 288.7; 422/65, 82.08, 100

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(57) **ABSTRACT**

An apparatus for automated preparation of DNA samples which comprises a reactor for preparing DNA samples and adjacent thereto an enzyme supply section, a plate holding section, a nozzle sealing section and a cleaning tank section, and wherein plates are loaded onto or unloaded from said plate holding section by means of a transport robot. The reactor for preparing DNA samples comprises a plurality of hollow electroconductive nozzles, hollow syringes coupled to said nozzles and pistons inserted into said syringes, the top of each of said pistons having a piston head secured thereto such that it can move up and down independently of said reactor for preparing DNA samples, the intermediate portions of said electroconductive nozzles being encased in a housing having an opening on both sides, a cooling mechanism being provided adjacent one of said openings, and electroconductive boards being connected to the intermediate portions of the electroconductive nozzles within said housing and also connected to a power supply via conductors.

31 Claims, 13 Drawing Sheets

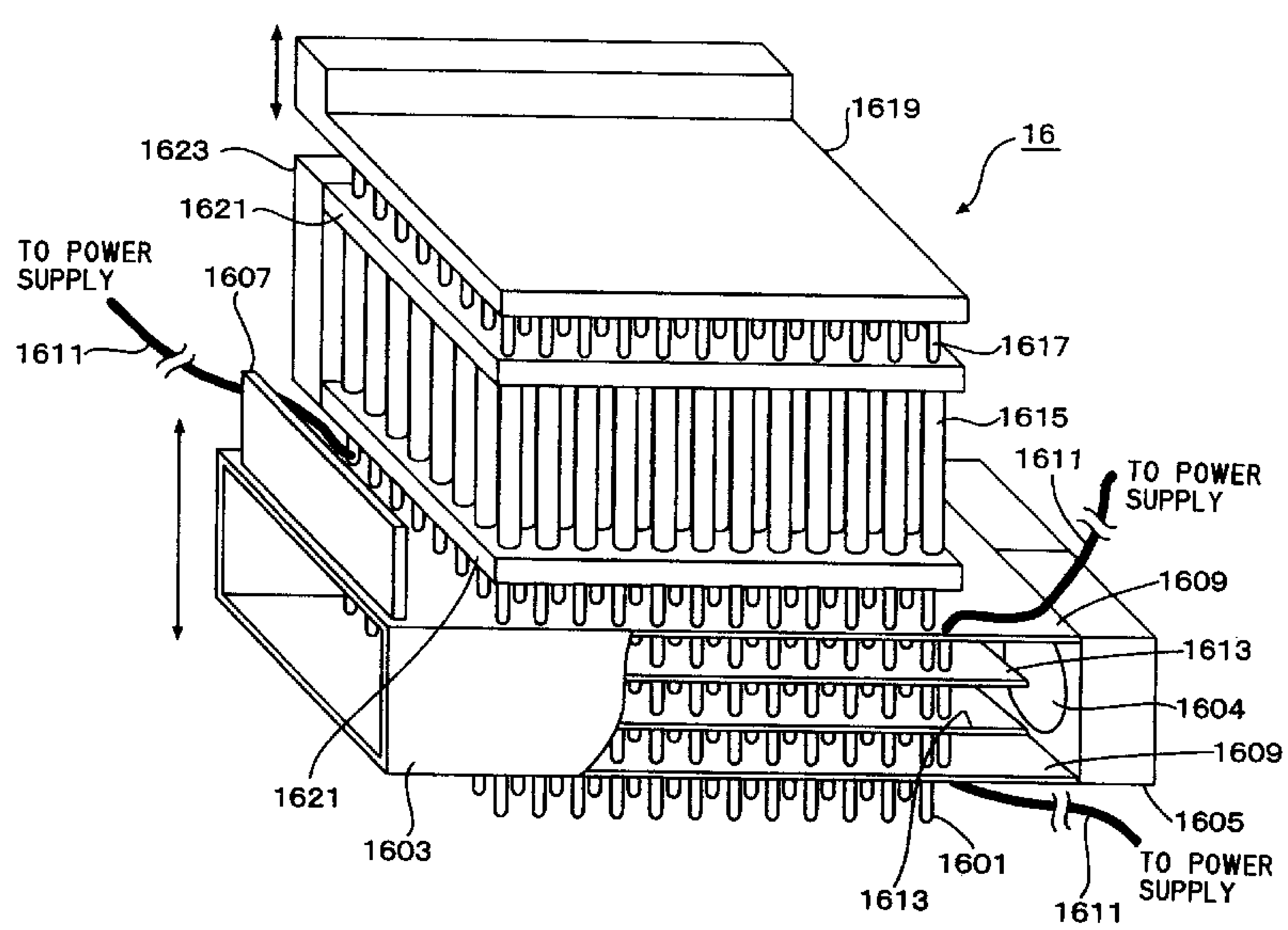


FIG.1

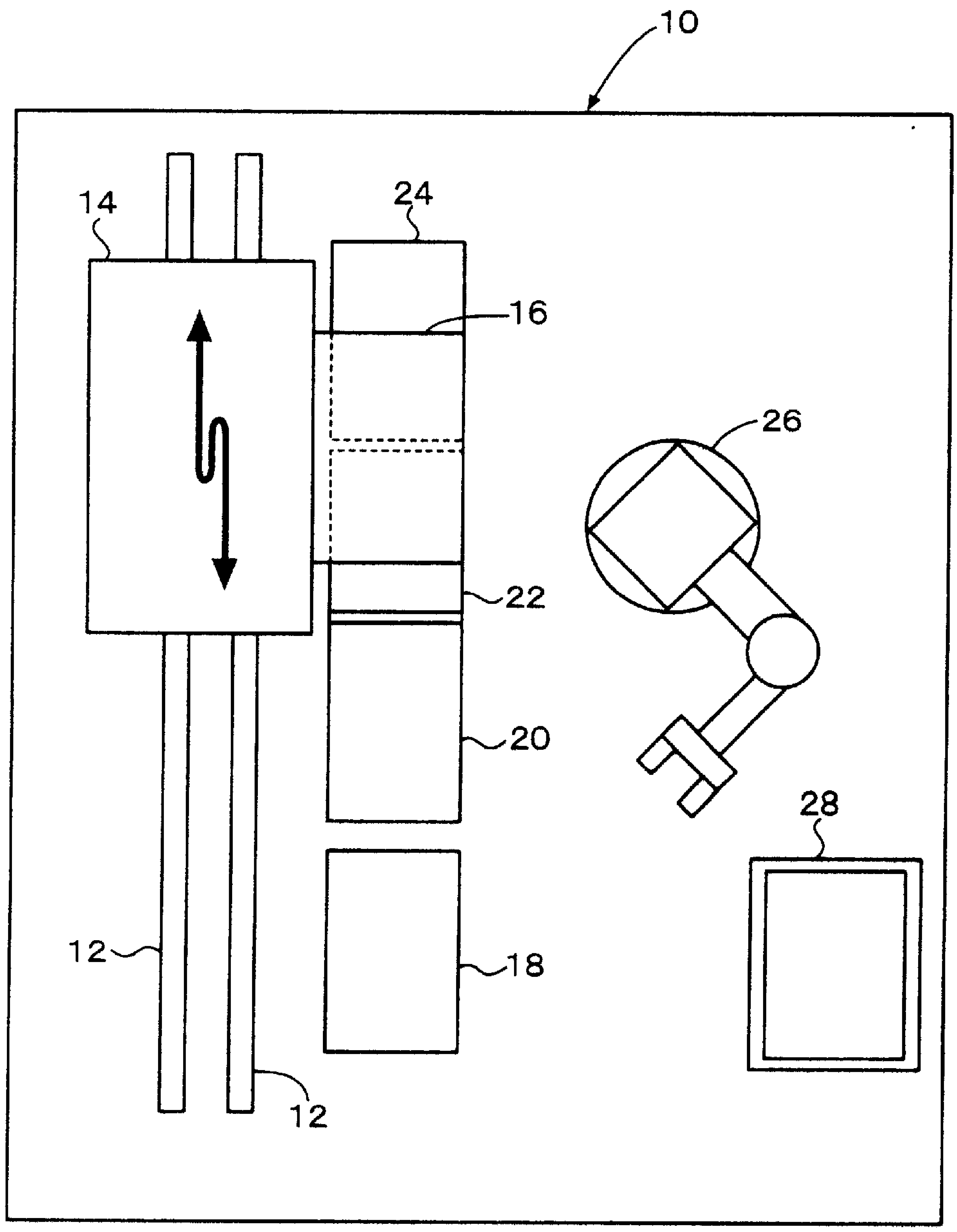


FIG.2

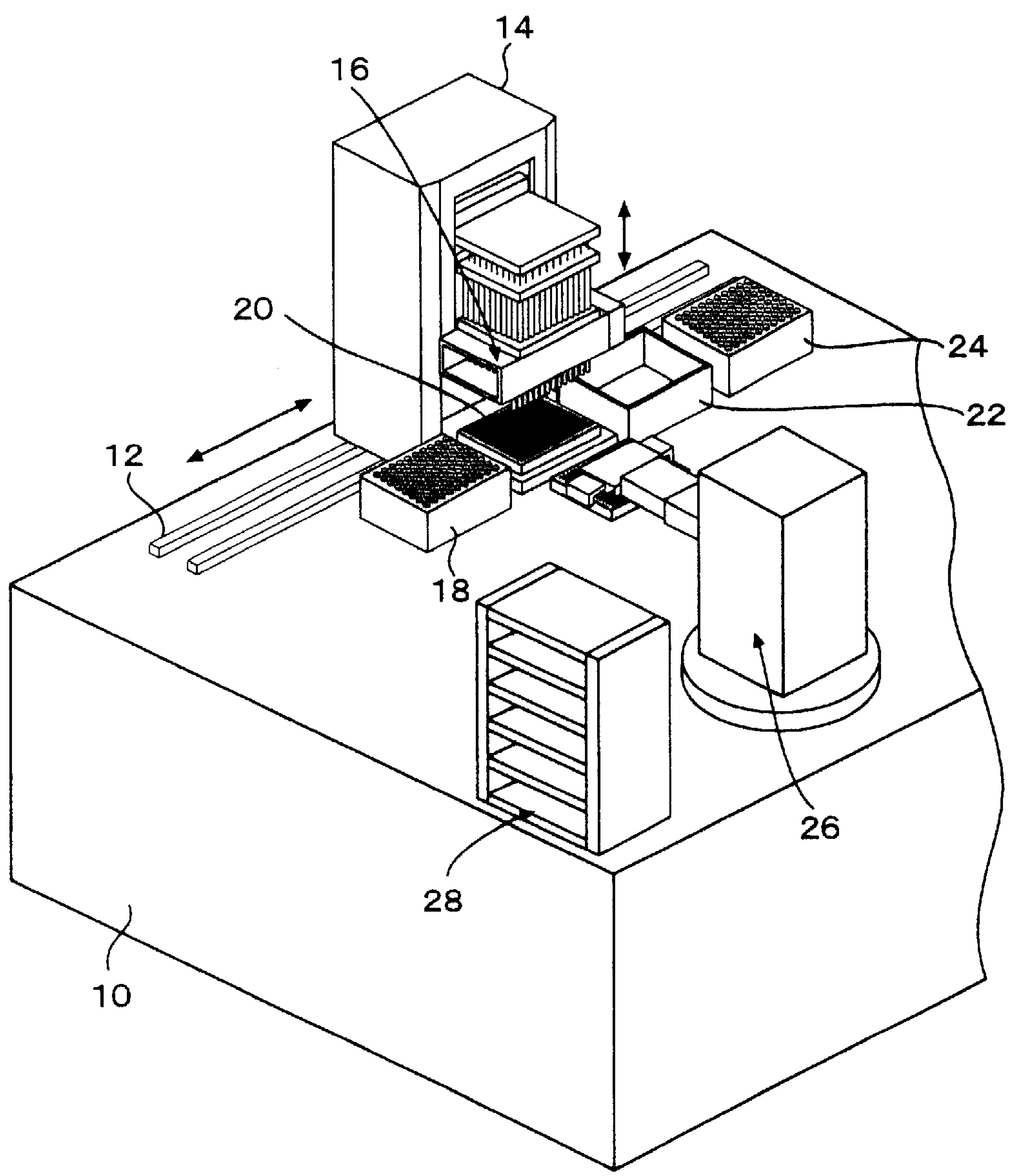


FIG.3

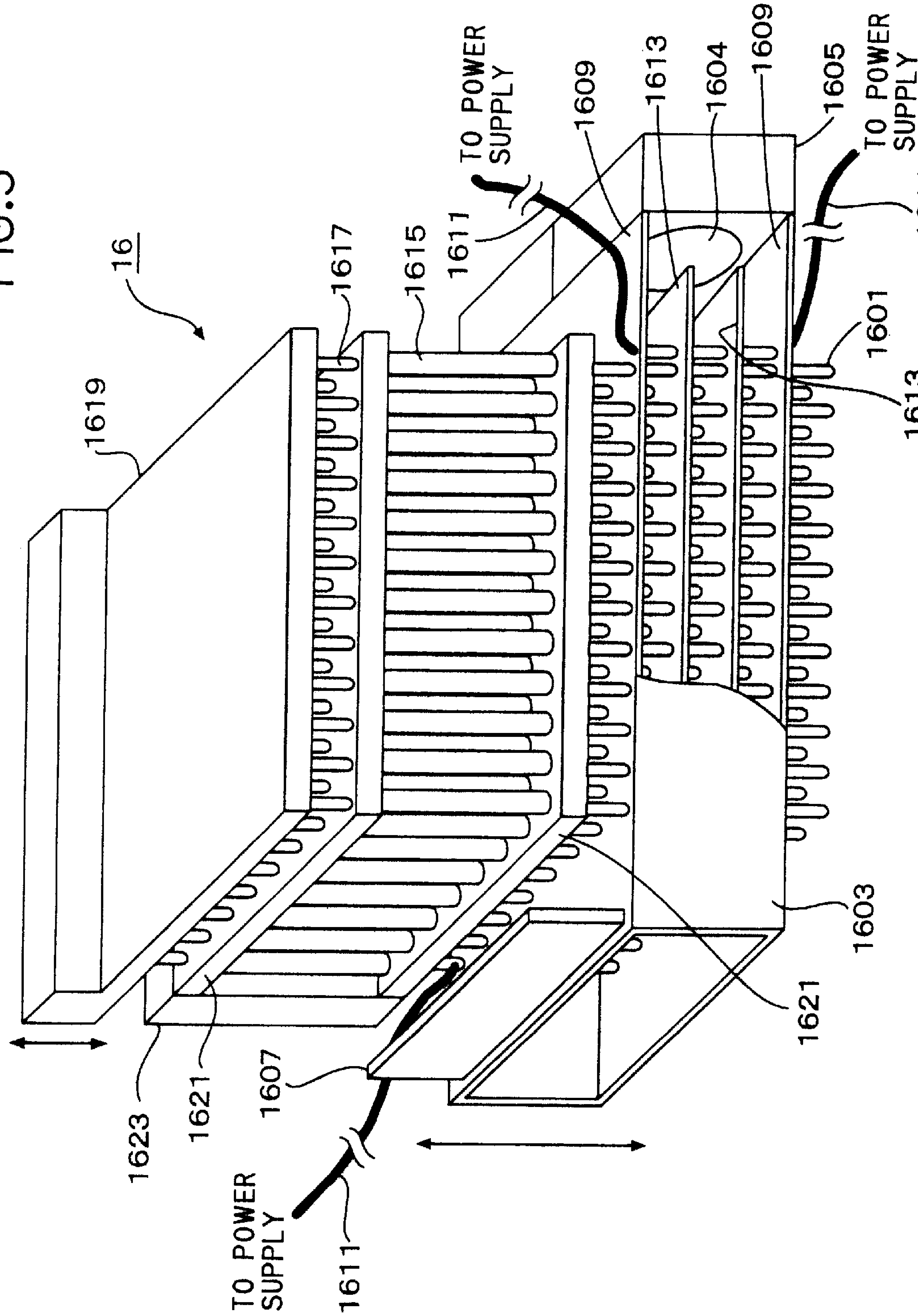

16
1619
1617
1615
1611
TO POWER SUPPLY
1609
1613
1604
1609
1605
TO POWER SUPPLY
1611
1601
1613
1603
1621
1621
1623
1607
TO POWER SUPPLY
1611

FIG.4

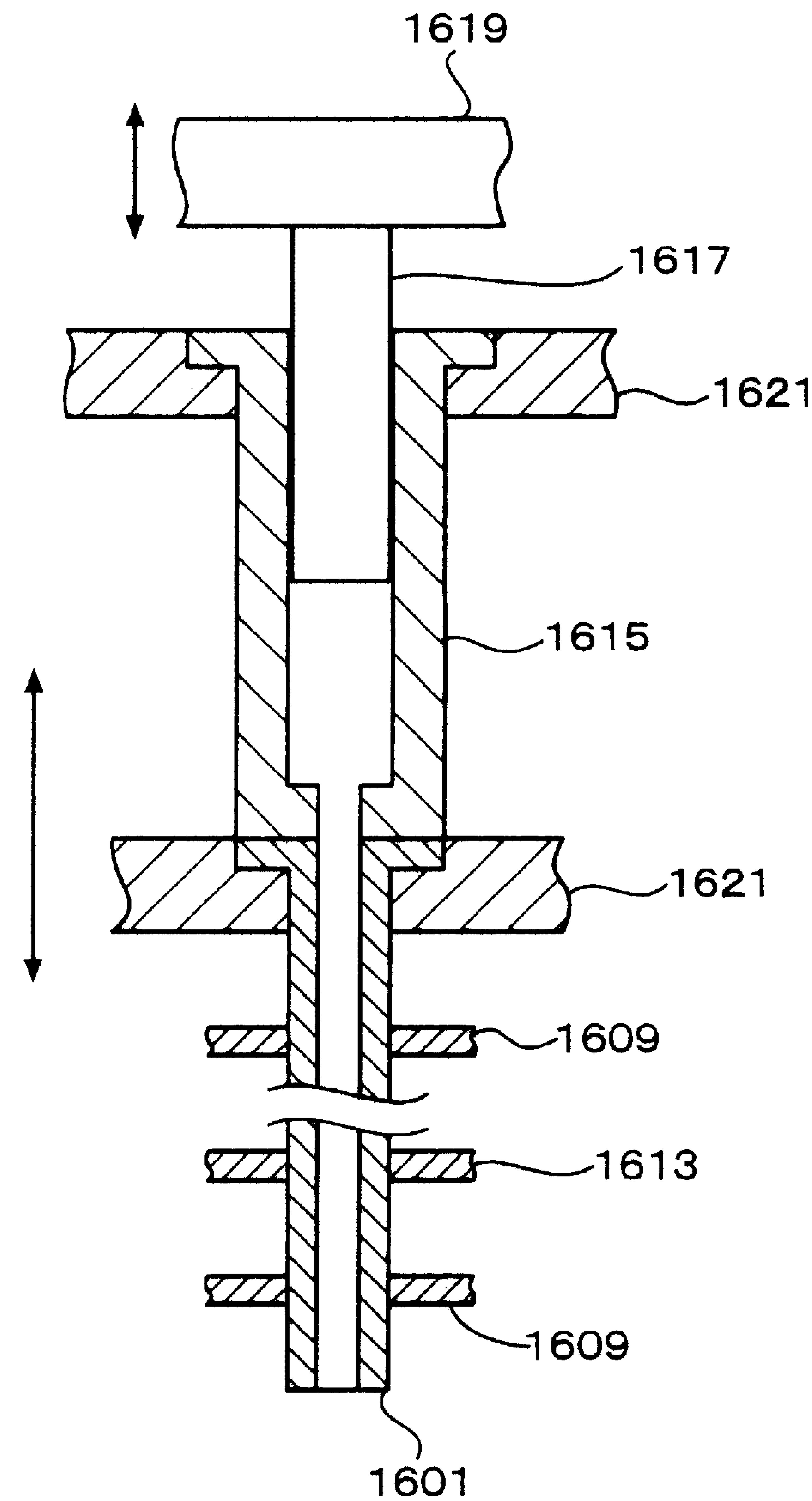


FIG.5

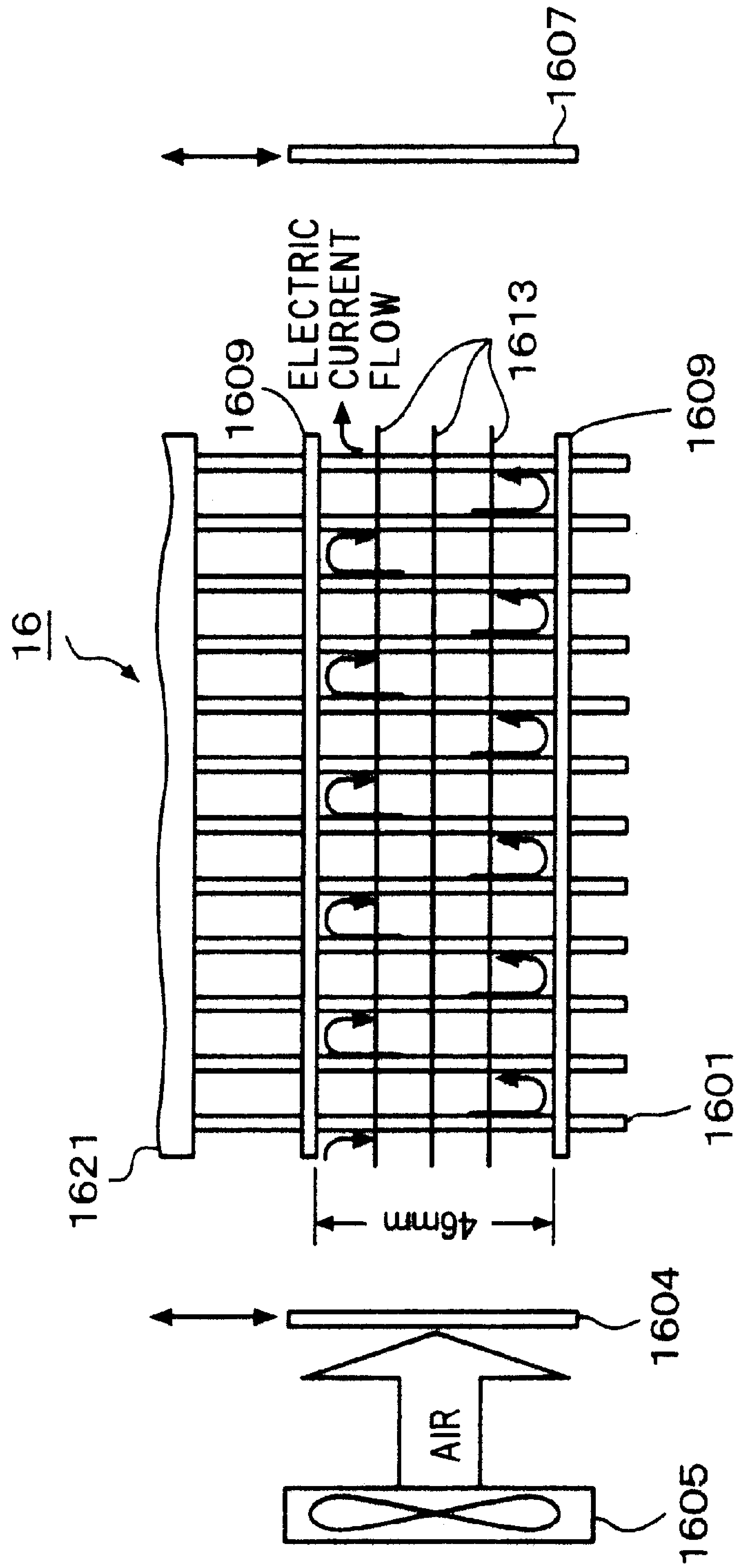

1607
ELECTRIC CURRENT FLOW
1613
1609
1609
16
1621
1601
46mm
1604
AIR
1605

FIG.6

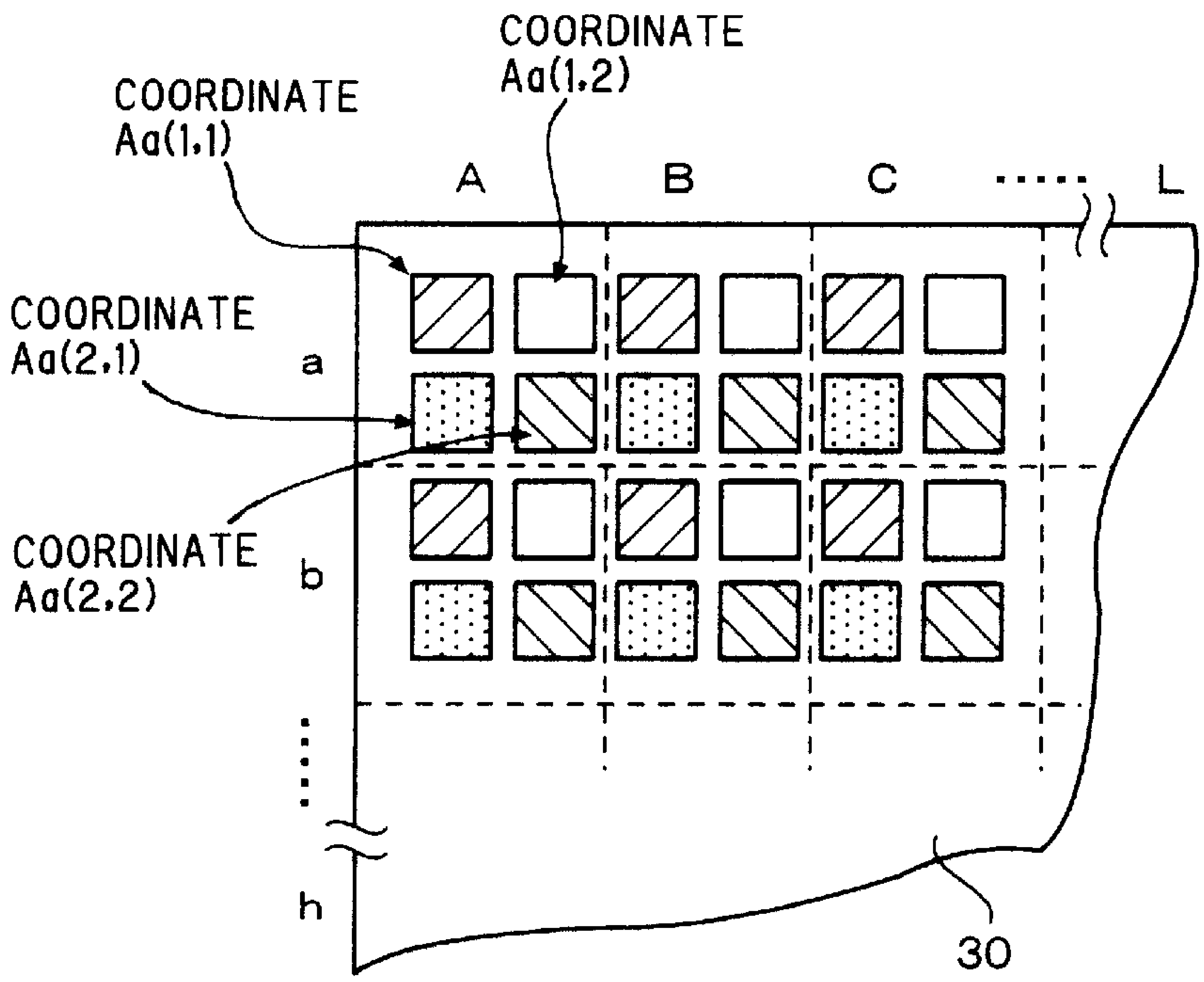

COORDINATE
Aa(1,2)
COORDINATE
Aa(1,1)
A
B
C
L
COORDINATE
Aa(2,1)
a
COORDINATE
Aa(2,2)
b
h
30

FIG.7

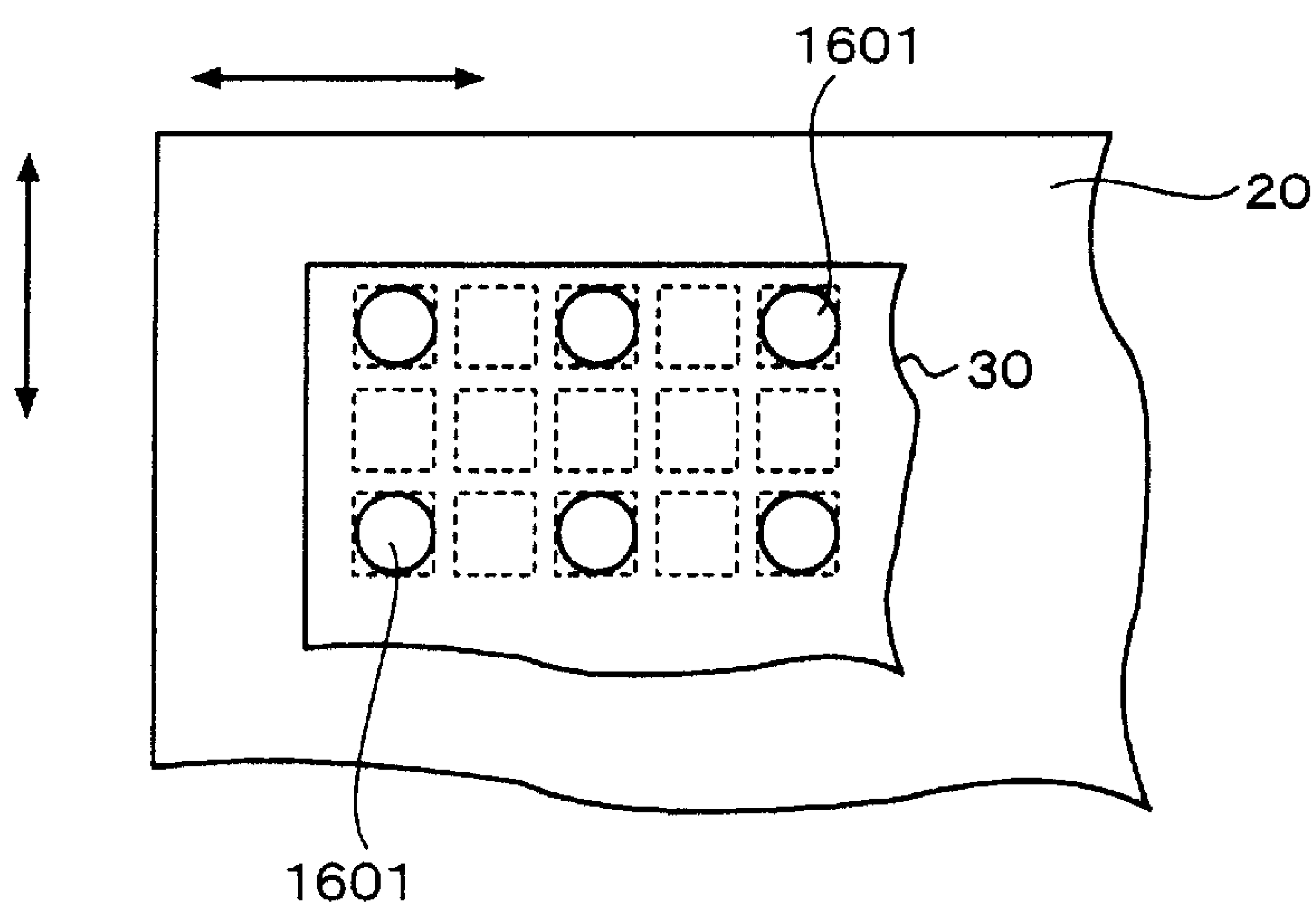
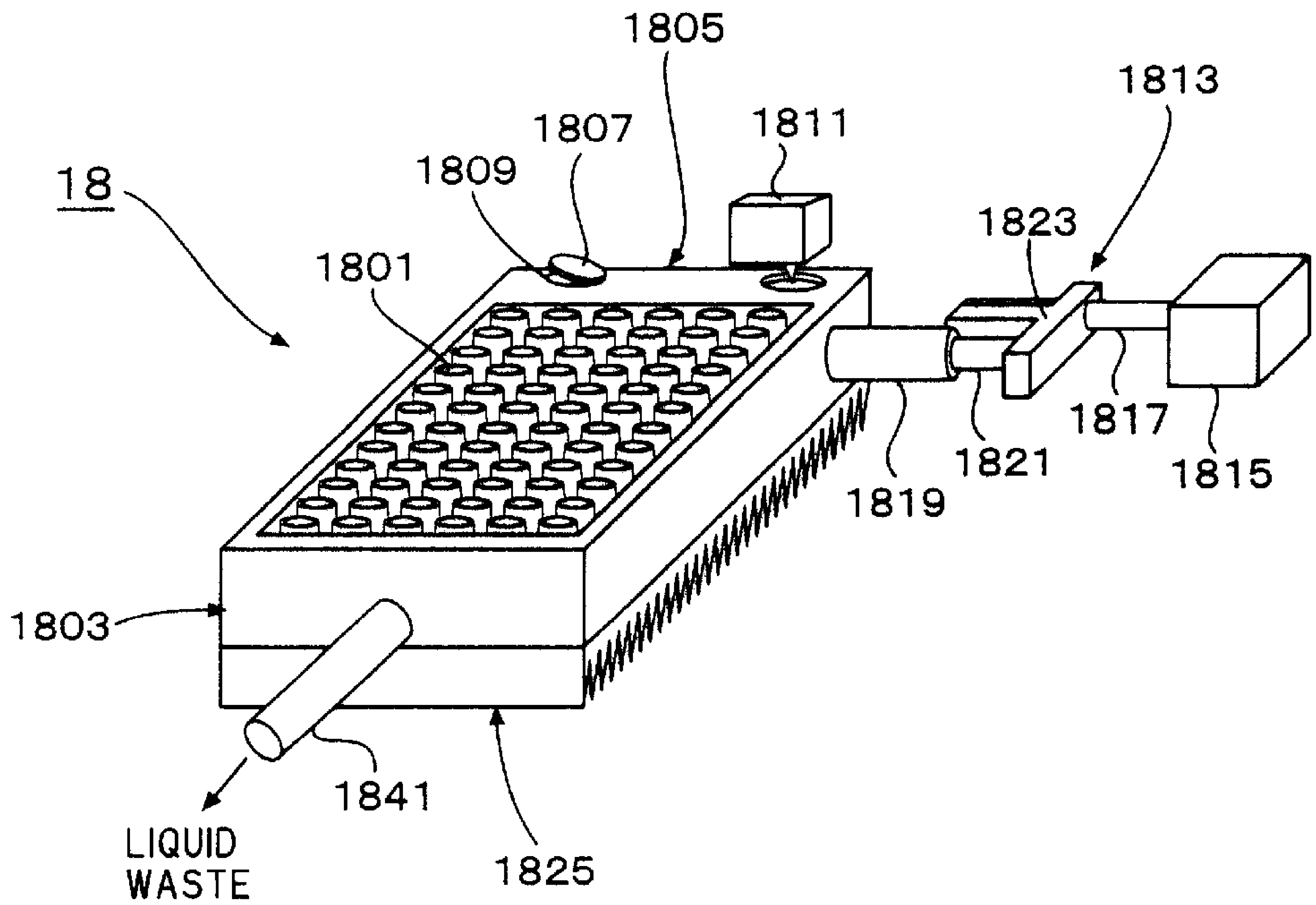
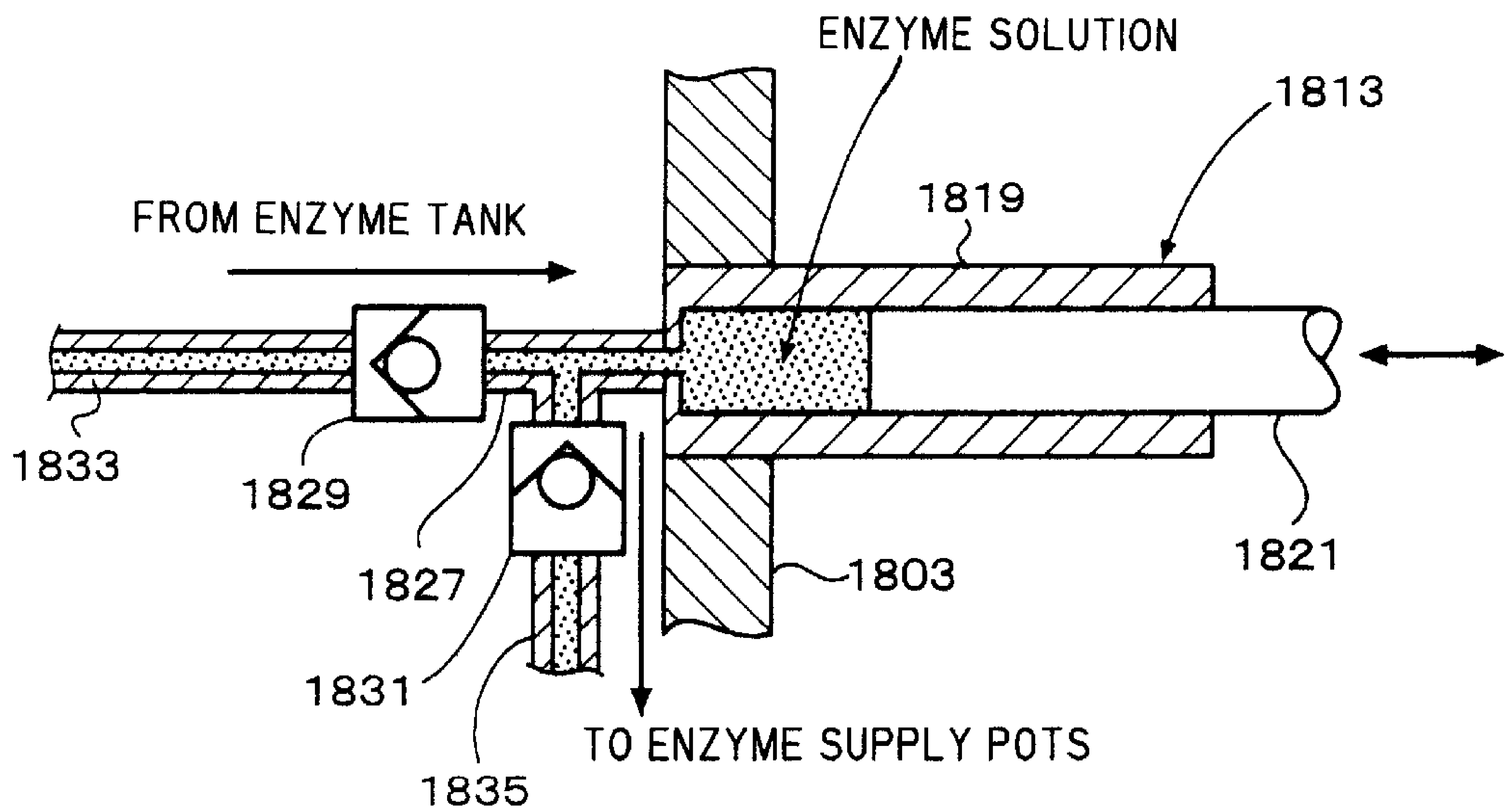

1601
20
30
1601

FIG.8



18
1805
1813
1807
1811
1809
1823
1801
1817
1821
1815
1819
1803
1841
1825
LIQUID WASTE

FIG.9



ENZYME SOLUTION
1813
1819
FROM ENZYME TANK
1833
1829
1827
1803
1821
1831
TO ENZYME SUPPLY POTS
1835

FIG.10

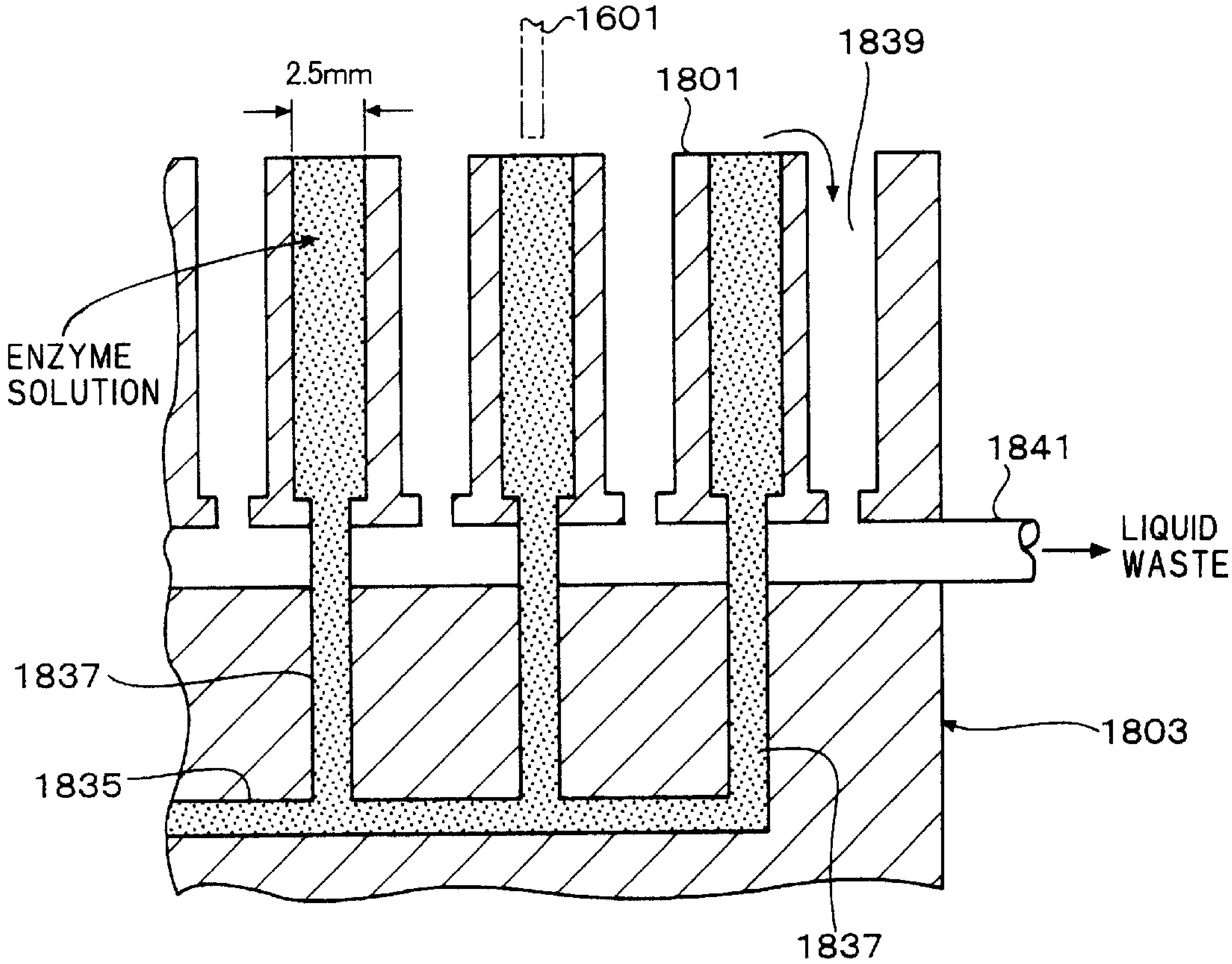

1601
1839
2.5mm
1801
ENZYME
SOLUTION
1841
LIQUID
WASTE
1837
1803
1835
1837

FIG.11

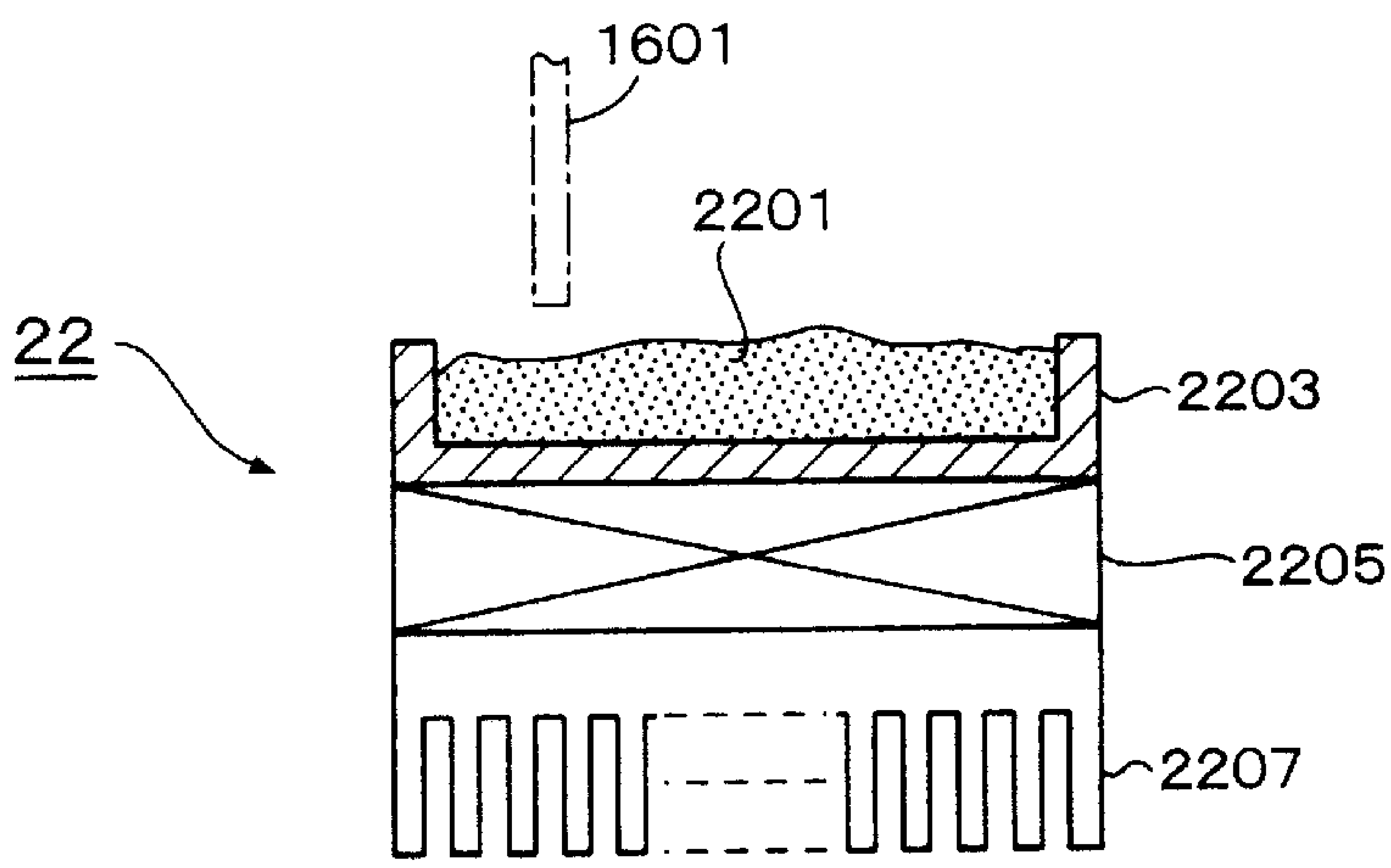

1601
2201
22
2203
2205
2207

FIG.12(A)

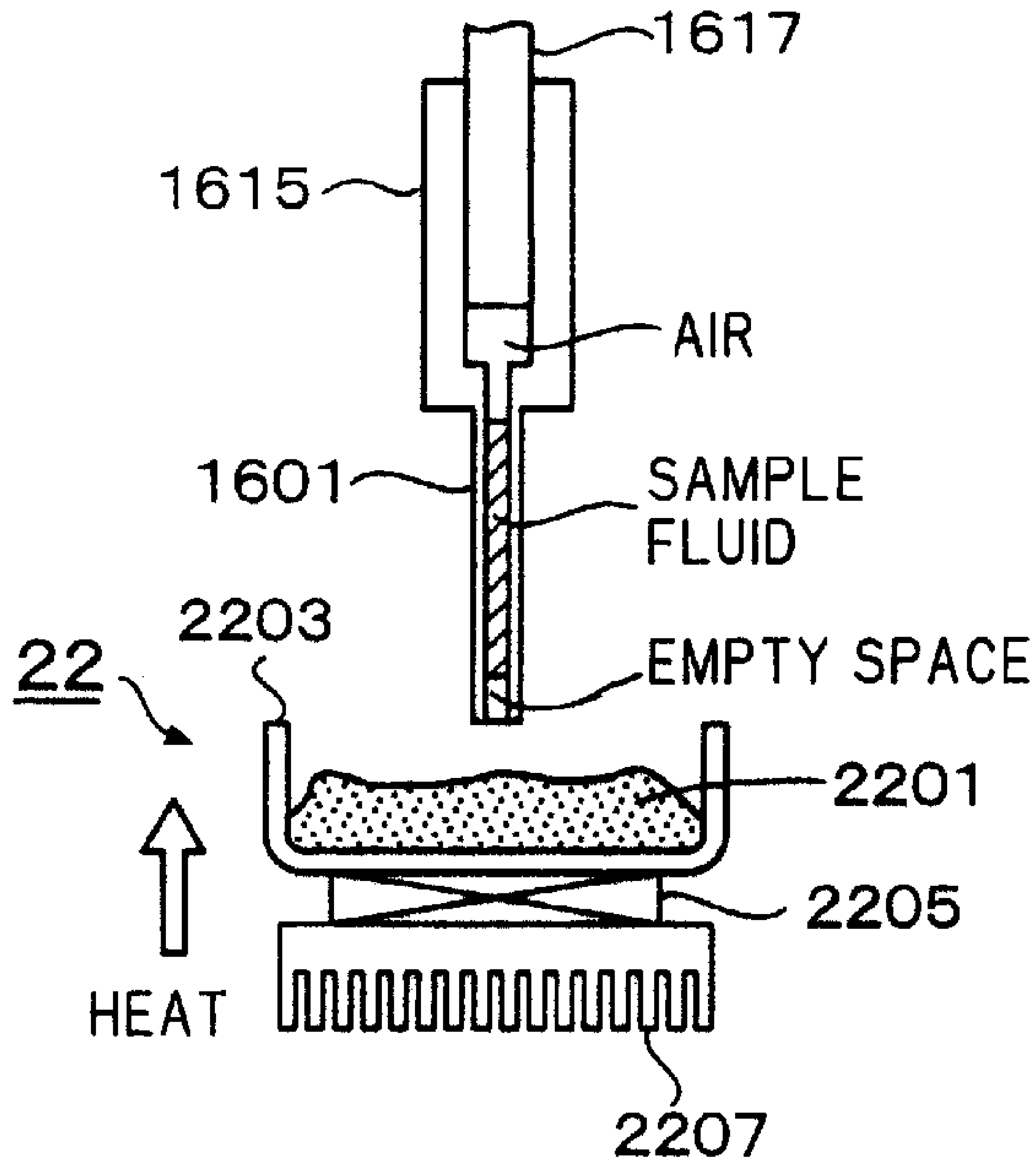


FIG.12(B)

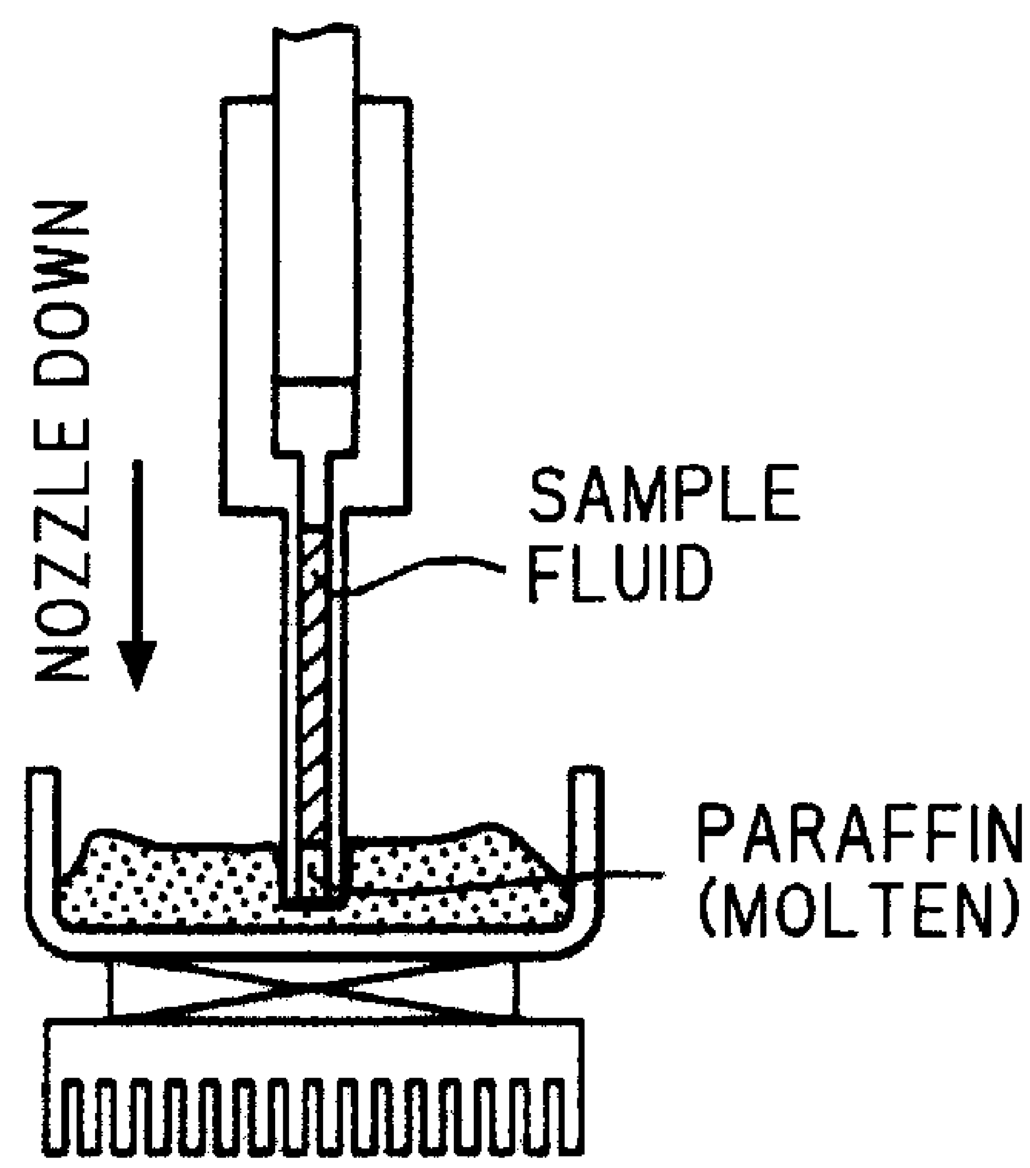


FIG.12(C)

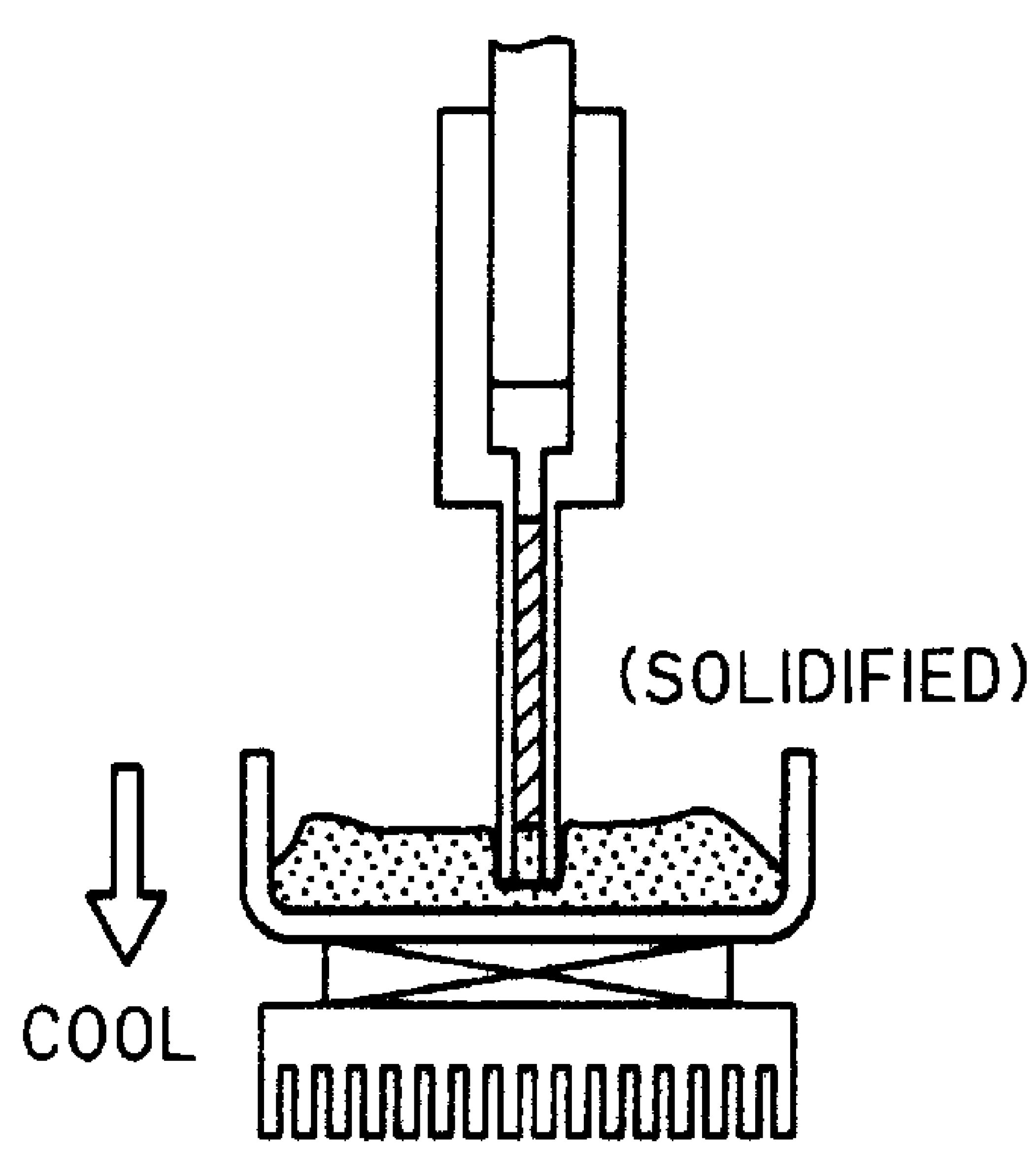


FIG.12(D)

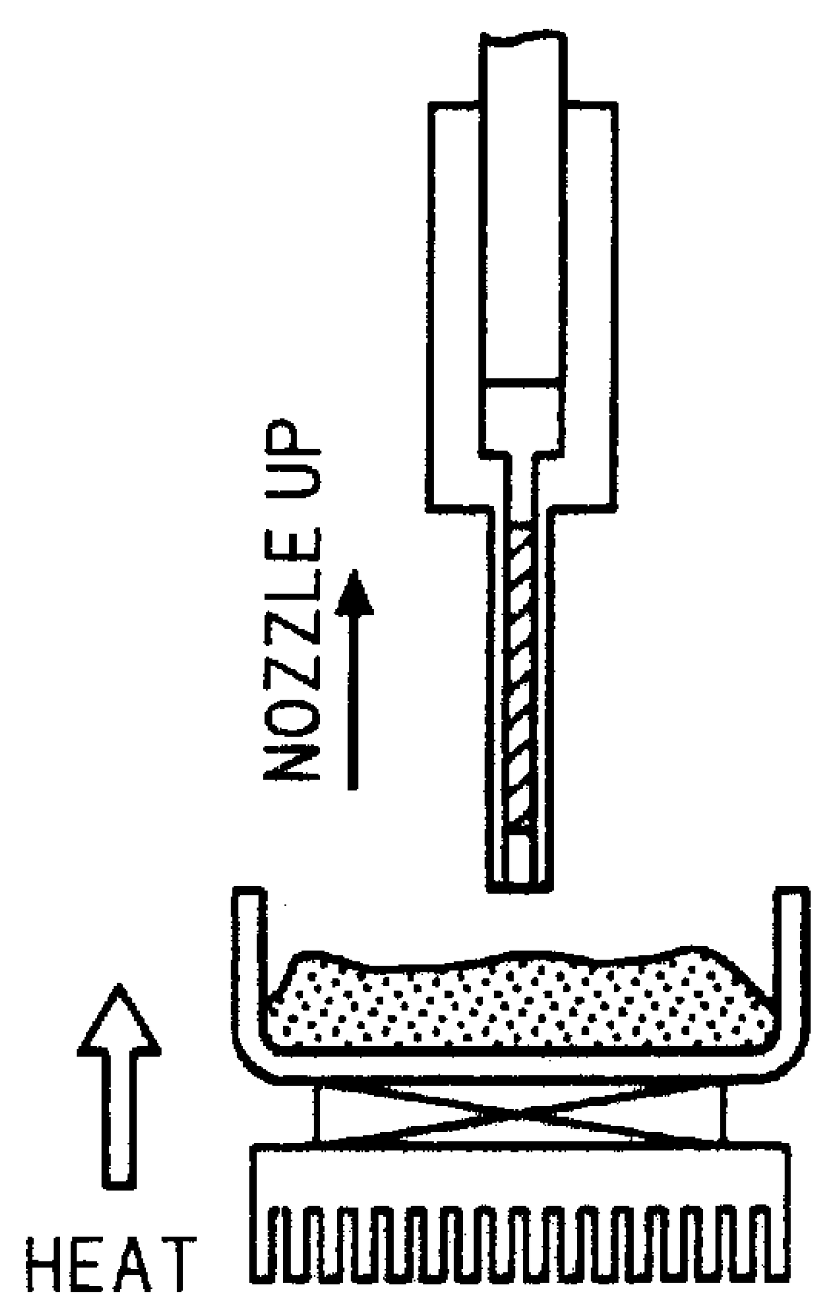


FIG.13

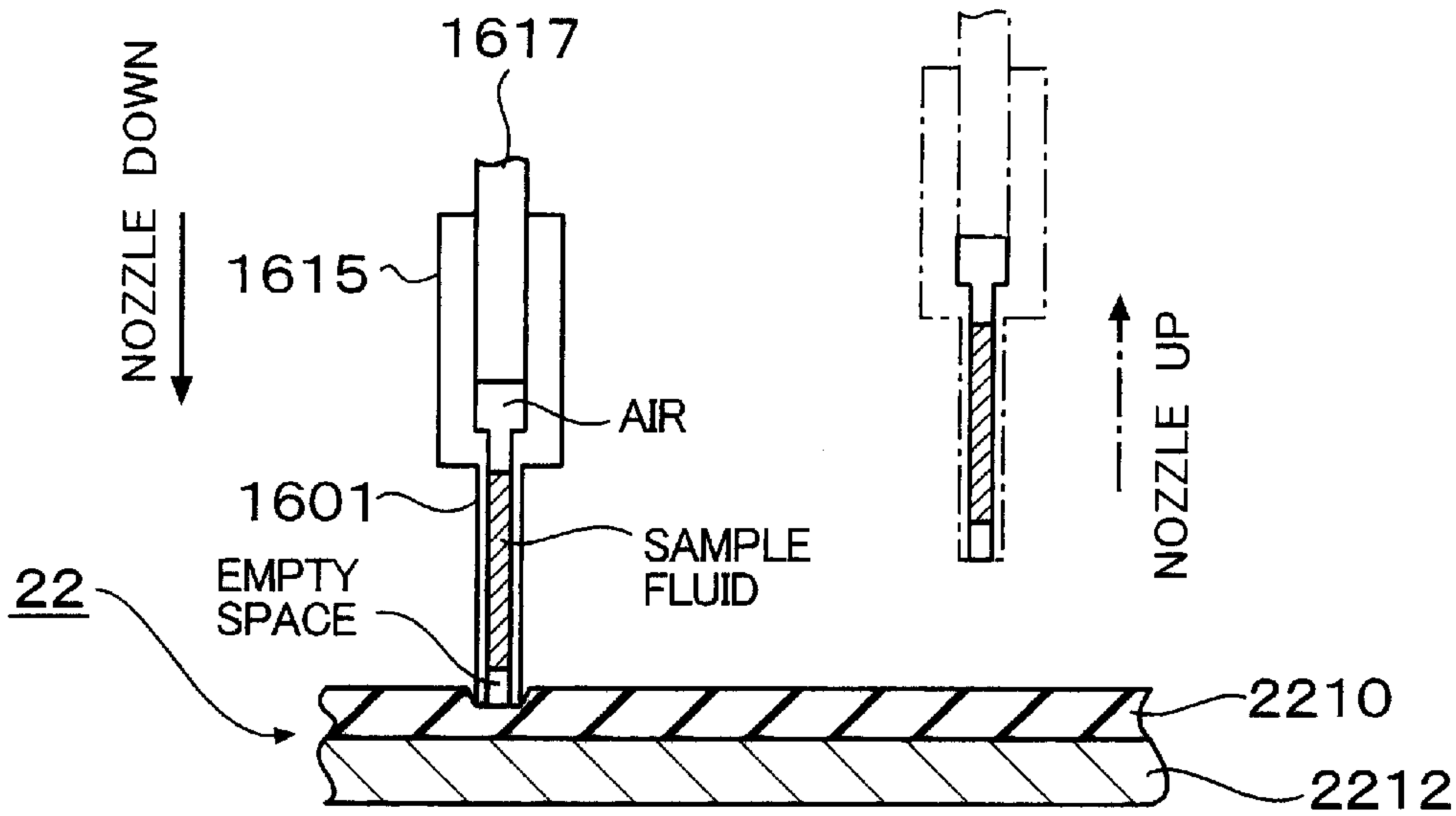

1617
NOZZLE DOWN
1615
AIR
1601
SAMPLE
FLUID
22
EMPTY
SPACE
NOZZLE UP
2210
2212

FIG.14

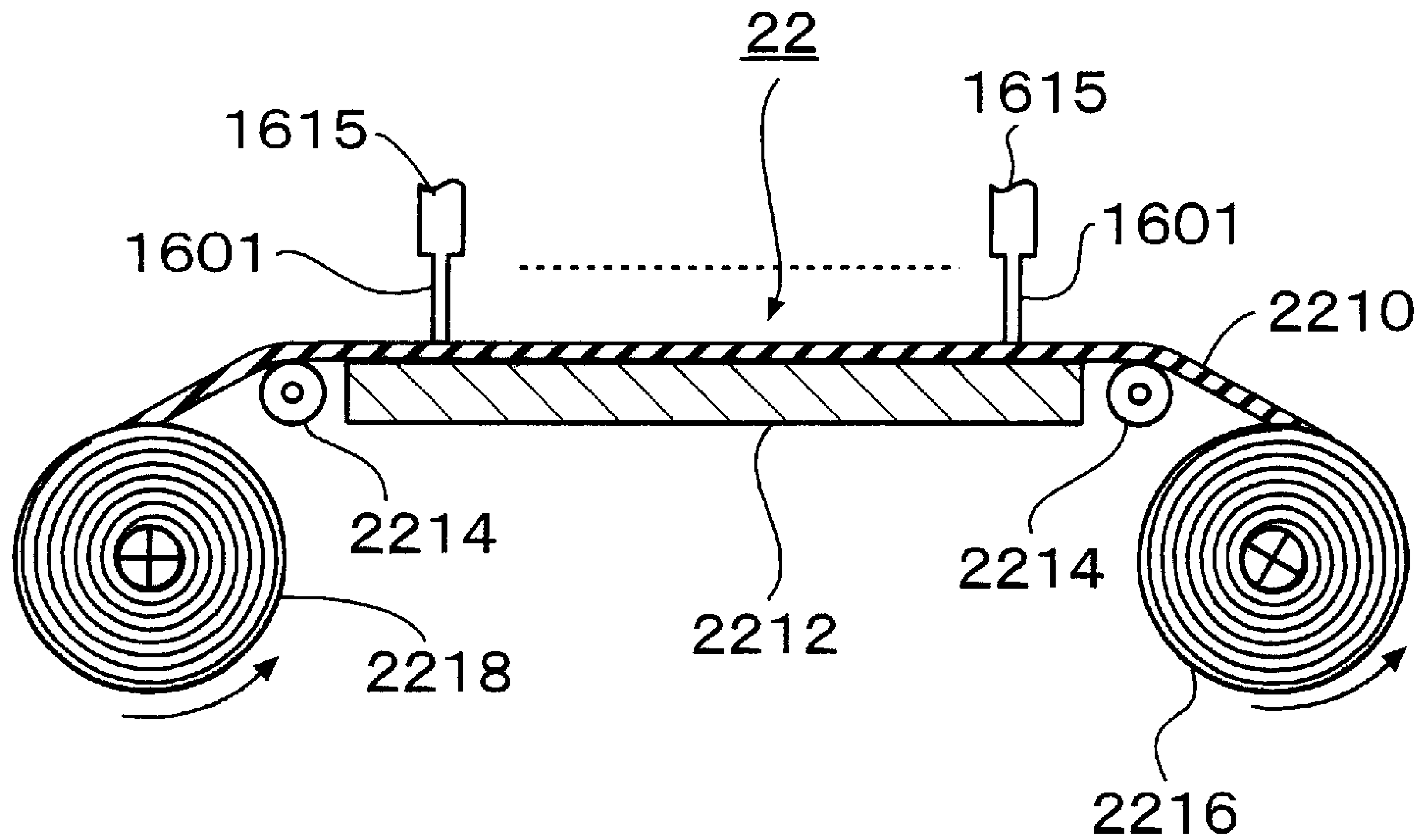

22
1615
1615
1601
1601
2210
2214
2214
2212
2218
2216

FIG.15

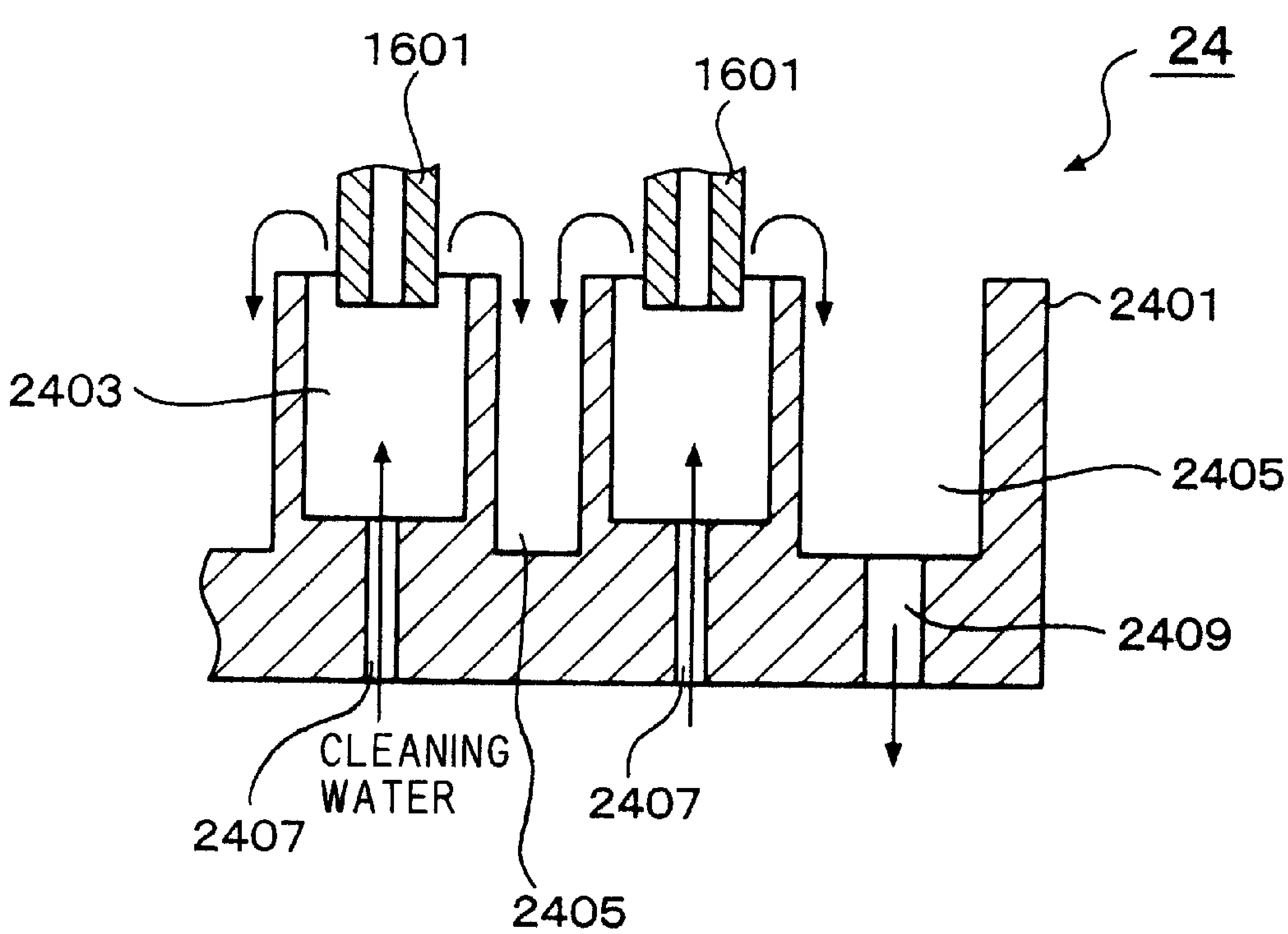

1601
1601
24
2401
2403
2405
2409
CLEANING
WATER
2407
2407
2405

FIG.16

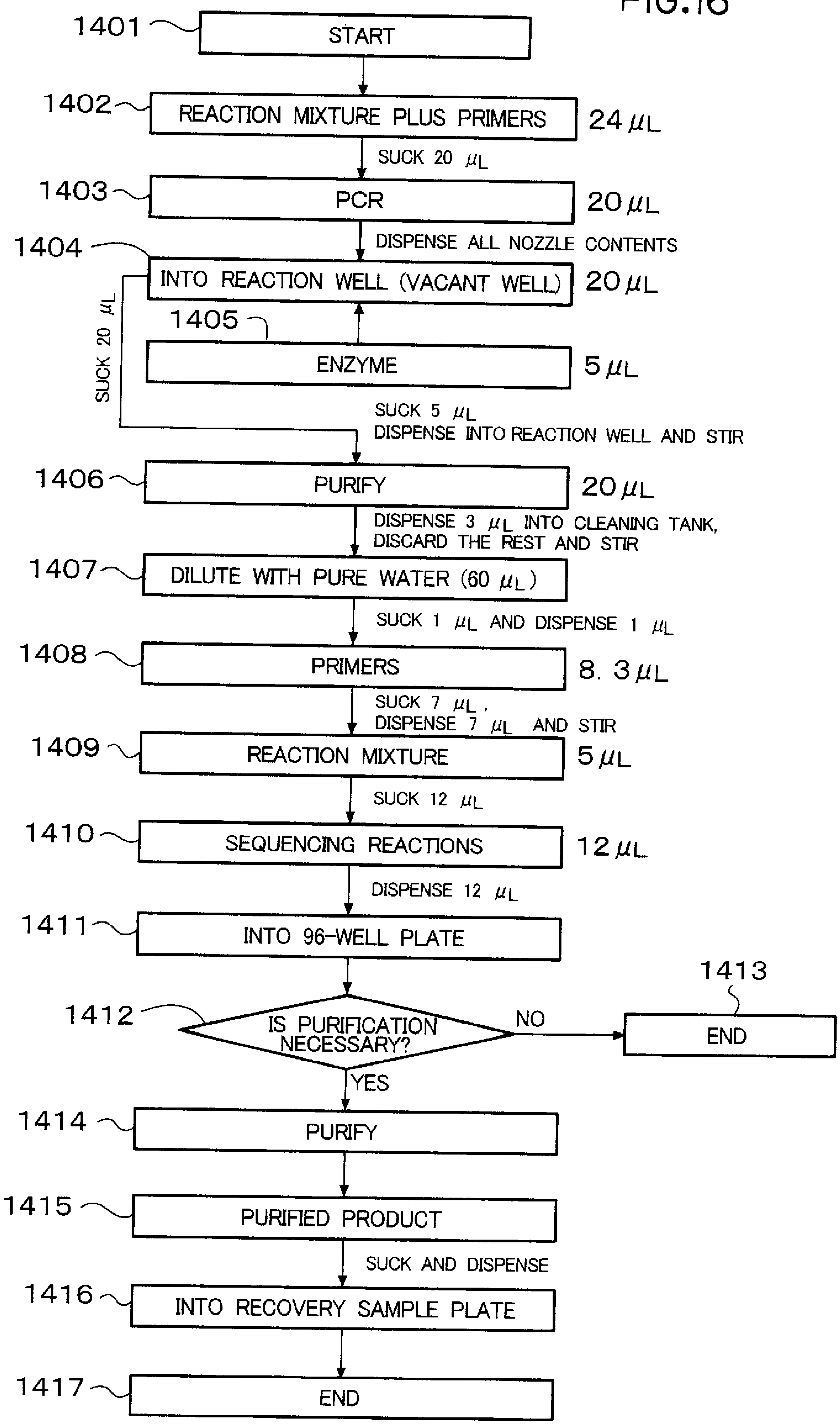
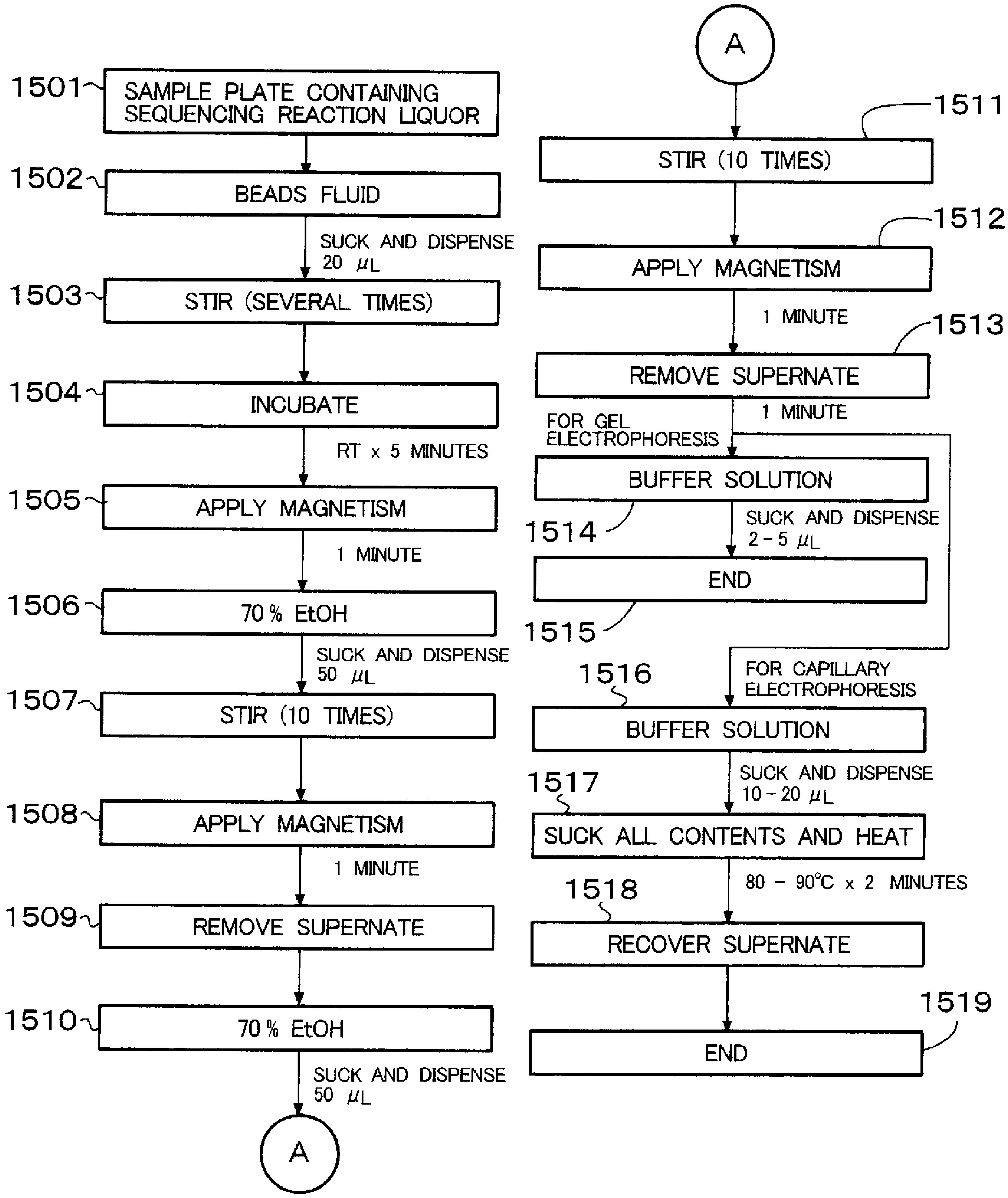

1401
START
1402
REACTION MIXTURE PLUS PRIMERS
24 μL
SUCK 20 μL
1403
PCR
20 μL
DISPENSE ALL NOZZLE CONTENTS
1404
INTO REACTION WELL (VACANT WELL)
20 μL
SUCK 20 μL
1405
ENZYME
5 μL
SUCK 5 μL
DISPENSE INTO REACTION WELL AND STIR
1406
PURIFY
20 μL
DISPENSE 3 μL INTO CLEANING TANK,
DISCARD THE REST AND STIR
1407
DILUTE WITH PURE WATER (60 μL)
SUCK 1 μL AND DISPENSE 1 μL
1408
PRIMERS
8. 3 μL
SUCK 7 μL,
DISPENSE 7 μL AND STIR
1409
REACTION MIXTURE
5 μL
SUCK 12 μL
1410
SEQUENCING REACTIONS
12 μL
DISPENSE 12 μL
1411
INTO 96-WELL PLATE
1412
IS PURIFICATION NECESSARY?
NO
1413
END
YES
1414
PURIFY
1415
PURIFIED PRODUCT
SUCK AND DISPENSE
1416
INTO RECOVERY SAMPLE PLATE
1417
END

FIG.17



1501 SAMPLE PLATE CONTAINING SEQUENCING REACTION LIQUOR
1502 BEADS FLUID
SUCK AND DISPENSE 20 μL
1503 STIR (SEVERAL TIMES)
1504 INCUBATE
RT x 5 MINUTES
1505 APPLY MAGNETISM
1 MINUTE
1506 70% EtOH
SUCK AND DISPENSE 50 μL
1507 STIR (10 TIMES)
1508 APPLY MAGNETISM
1 MINUTE
1509 REMOVE SUPERNATE
1510 70% EtOH
SUCK AND DISPENSE 50 μL
A
A
1511 STIR (10 TIMES)
1512 APPLY MAGNETISM
1 MINUTE
1513 REMOVE SUPERNATE
1 MINUTE
FOR GEL ELECTROPHORESIS
1514 BUFFER SOLUTION
SUCK AND DISPENSE 2-5 μL
1515 END
FOR CAPILLARY ELECTROPHORESIS
1516 BUFFER SOLUTION
SUCK AND DISPENSE 10-20 μL
1517 SUCK ALL CONTENTS AND HEAT
80 - 90°C x 2 MINUTES
1518 RECOVER SUPERNATE
1519 END

APPARATUS FOR AUTOMATED PREPARATION OF DNA SAMPLES AND REACTOR FOR PREPARING DNA SAMPLES

BACKGROUND OF THE INVENTION

This invention relates to an apparatus for automated preparation of DNA samples that are to be analyzed with a DNA sequencer. More particularly, the invention relates to an automated apparatus with which fluorescence-labelled DNA samples can be prepared with the aid of amplification by thermal cycling, in particular, amplification by polymerase chain reaction (PCR).

A specified DNA region can be amplified at least about 10^5-fold by repeating DNA synthesis reactions using two primers bracketing that region and a DNA synthetase. This method called polymerase chain reaction (PCR) was developed by the American company Cetus in 1985. It was later modified to use thermostable Taq polymerase and has been established as an efficient PCR-based method. In 1987, another American company Perkin-Elmer and Cetus jointly developed an automated PCR apparatus which contributed to a marked growth of the use of PCR.

Details about the theory of PCR are given in many prior art references. According to Jikken Igaku (Experimental Medicine), Vol. 7, No. 2, pp. 14–18 (1989), the PCR is the sequence of the following three steps: (1) duplex DNA to which primers are joined is thermally denatured to single strands, (2) the primers are annealed and (3) the primed DNA is extended by Taq polymerase. The cycle is repeated several tens of times to amplify the desired DNA fragment.

In the conventional automated PCR apparatus, polymerase chain reaction has been carried out in small test tubes such as plastic Eppendorf tubes which are placed within a chamber that permits temperature control. By changing the temperature in this chamber, the temperature of the reaction mixtures in the Eppendorf tubes is cyclically changed to the levels necessary for (1) denaturation (ca. 94° C.), (2) annealing (ca. 55° C.) and (3) extension (ca. 72° C.).

The conventional automated PCR apparatus can yield only PCR products and their purification, sequencing reaction (for processing the PCR products to prepare samples that can be analyzed with a DNA sequencer) and the purification of the sequencing reaction product cannot be accomplished without a separate apparatus or manual operations.

SUMMARY OF THE INVENTION

An object, therefore, of the invention is to provide an apparatus for automated preparation of DNA samples by which an entire process comprising the preparation of PCR products, their purification, sequencing reaction and the purification of the sequencing reaction product can be performed automatically.

This object of the invention can be attained by an apparatus for automated preparation of DNA samples which comprises a reactor for preparing DNA samples and adjacent thereto an enzyme supply section, a plate holding section, a nozzle sealing section and a cleaning tank section, and wherein plates are loaded onto or unloaded from said plate holding section by means of a transport robot.

Said reactor for preparing DNA samples is supported on a unidirectionally moving mechanism to be capable of moving up and down and comprises a plurality of hollow electroconductive nozzles, hollow syringes coupled to said nozzles and pistons inserted into said syringes, the top of each of said pistons having a piston head secured thereto such that it can move up and down independently of said reactor for preparing DNA samples, the intermediate portions of said electroconductive nozzles being encased in a housing having an opening on both sides, a cooling mechanism being provided adjacent one of said openings, and electroconductive boards being connected to the intermediate portions of the electroconductive nozzles within said housing and also connected to a power supply via conductors.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a simplified plan view showing an exemplary layout of the apparatus of the invention for automated preparation of DNA samples;

FIG. **2** is a simplified perspective view of the same apparatus;

FIG. **3** is a simplified perspective view showing, with part taken away, the reactor for preparing DNA samples which is used in the apparatus of the invention for automated preparation of DNA samples;

FIG. **4** is a simplified partial sectional view showing how nozzles, syringes and pistons are assembled for integration in the reactor shown in FIG. **3**;

FIG. **5** is a schematic simplified view showing how the nozzles in the reactor shown in FIG. **3** are heated and cooled;

FIG. **6** is a partial enlarged schematic view of a plate to be used in the apparatus of the invention for automated preparation of DNA samples;

FIG. **7** is a partial enlarged schematic view showing the plate of FIG. **6** into which some nozzles have been inserted;

FIG. **8** is a simplified perspective view of the enzyme supply section which is used in the apparatus of the invention for automated preparation of DNA samples;

FIG. **9** is a partial enlarged section of the solution transfer mechanism provided in the enzyme supply section shown in FIG. **8**;

FIG. **10** is a partial enlarged section of enzyme supply pots provided in the enzyme supply section shown in FIG. **8**;

FIG. **11** is a simplified sectional view showing an exemplary structure of the nozzle sealing section which is used in the apparatus of the invention for automated preparation of DNA samples;

FIG. **12**A shows schematically a nozzle in the nozzle sealing section which is about to be inserted into a heated paraffin bath;

FIG. **12**B shows schematically the nozzle as it has been inserted into molten paraffin;

FIG. **12**C shows schematically the nozzle as it has been sealed by solidified paraffin;

FIG. **12**D shows schematically the nozzle as it has been withdrawn from remelted paraffin;

FIG. **13** is a simplified sectional view showing another exemplary structure of the nozzle sealing section which is used in the apparatus of the invention for automated preparation of DNA samples;

FIG. **14** is a simplified sectional view showing an embodiment in which a continuous web of sheet is used as the mat in the nozzle sealing section shown in FIG. **13**;

FIG. **15** is a partial simplified sectional view showing an exemplary structure of the cleaning tank section which is used in the apparatus of the invention for automated preparation of DNA samples;

FIG. **16** is a flowchart for the steps in the process of performing PCR and sequencing reactions by the apparatus of the invention for automated preparation of DNA samples; and

FIG. **17** is a flowchart for an exemplary process of purification that is performed in step **1414** in the flowchart of FIG. **16**.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The apparatus of the invention for automated preparation of DNA samples is described below more specifically with reference to the accompanying drawings. FIG. **1** is a simplified plan view showing an exemplary layout of the apparatus. It comprises a horizontal bench **10** carrying two parallel guide rails **12** over which a moving mechanism **14** rests. The moving mechanism **14** is adapted to move along the guide rails **12** in one direction either back and forth or to right and left. A reactor **16** for preparing DNA samples (see below) is fitted to the moving mechanism **14**. Parallel to the guide rails **12** are provided an enzyme supply section **18**, a plate holding section **20**, a nozzle sealing section **22** and a cleaning tank section **24** that are aligned in a straight line. Plates are loaded onto and/or unloaded from the plate holding section **20** by means of a transport robot **26**. The plates are if housed in a plate stacker **28**.

FIG. **2** is a simplified perspective view of the apparatus shown in FIG. **1**. The plate stacker **28** has a plurality of horizontal trays placed one above another and a supply plate or a recovery plate is to be put on each tray. If desired, two dedicated plate stackers may be used, one for supply plates and the other for recovery plates. To start processing, the transport robot **26** picks up a supply plate from one of the trays in the plate stacker **28**, carries it to the plate holding section **20** and places it in a predetermined position. The reactor **16** for preparing DNA samples is fitted to the moving mechanism **14** such that it can be moved up and down. The vertically moving mechanism for the reactor **16** may be of a known conventional type such as a stepping motor, a hydraulic mechanism or a ball screw mechanism. If a new plate is put in the plate holding section **20**, the moving mechanism **14** moves to this section, moves the apparatus **16** up and down, sucks reagents from within predetermined wells in the plate, further moves to appropriate positions such as the enzyme supply section **18**, nozzle sealing section **22** and cleaning tank sections **24** in accordance with a programmed routine, and performs predetermined processing operations which will be described below in detail. When all steps of processing are executed, the transport robot **26** picks up the plate from the plate holding section **20** and puts it back onto a tray for recovery plate in the plate stacker **28**.

The operating panel for manipulating the apparatus of the invention for automated preparation of DNA samples, the control unit loaded with programs for controlling various processing actions and other necessary equipment are not shown in FIGS. **1** and **2** but it will be readily understood by the skilled artisan that such operating panel, control unit and equipment are provided as necessary. Needless to say, the horizontal bench **10** of the apparatus of the invention for automated preparation of DNA samples may be covered with a suitable hermetic hood which serves as an effective means for preventing samples and reagents from being contaminated by dust, dirt and suspended miscellaneous germs in air.

FIG. **3** is a simplified perspective view of the reactor **16** which is used in the apparatus of the invention for automated preparation of DNA samples. The reactor **16** has nozzles **1601** extending from the bottom that are made of a conductive material, say, stainless steel. The nozzles **1601** are typically **96** in number but their number is variable. The intermediate portions of the nozzles **1601** are encased in a housing **1603** that can be rendered hermetic. At one end of the housing **1603** is provided a cooling mechanism (e.g. air-cooled fan) **1605** having a shutter **1064** that can be slid open. A shutter **1607** that can be slid open is also provided at the other end of the housing **1603**. Nozzles **1601** are connected to two conductive boards **1609**, one in the upper part and the other in the lower part. The conductive boards **1609** are typically made of copper or stainless steel. The area defined by the two conductive boards **1609** is the heating zone for the nozzles **1601**. The housing **1603** may remain open at both ends.

In this case, there is no need to provide the shutters **1604** and **1607** but the air-cooled fan **1605** without shutter is provided adjacent one open end of the housing **1603**.

Conductor cables **1611** extending from a power supply (not shown) are connected to the conductive boards **1609**. If the power supply is turned on, an electric current flows from the conductive boards **1609** to the individual conductive nozzles **1601**, which are heated by Joule's heat. Conversely, if the power supply is turned off, the heating of the nozzles **1601** ends and both the shutter **1604** on the air-cooled fan **1605** and the shutter **1607** are slid open and the air-cooled fan **1605** is driven to cool the nozzles **1601**. Plates **1613** are provided between the conductive boards **1609** for straightening the air streams created by the fan **1605** so that the nozzles **1601** are effectively cooled within a short time. Two straightening plates are shown in FIG. **3** but three or more straightening plates may be provided. The straightening plates **1613** are typically made of ceramics, copper, plastics or stainless steel.

The nozzles **1601** are coupled to syringes **1615**. Pistons **1617** are inserted into the syringes and a vertically movable piston head **1619** is securely fixed to the pistons **1617** at the top. The piston head **1619** is supported on an independent vertically moving mechanism (not shown) which can be operated independently of the vertically moving mechanism for the overall operation of the reactor **16**. The vertically moving mechanism for the piston head **1619** may also be of a known conventional type such as a stepping motor, a hydraulic mechanism or a ball screw mechanism.

The syringes **1615** are fixed to two fixing plates **1621**, one at the top and the other on the bottom. The fixing plates **1621** are securely fixed to a mounting plate **1621** at an end. The mounting plate **1621** is attached to the vertically moving mechanism (not shown) in the moving mechanism **14** so as to secure the vertical movements of the reactor **16**. As already mentioned, the piston head **1619** is attached to the other independent vertically moving mechanism (not shown) in the moving mechanism **14** so as to secure the vertical movements of the pistons **1617**.

FIG. **4** is a partial enlarged sectional view showing how nozzles **1601**, syringes **1615**, pistons **1617** and piston head **1619** are assembled as an integral unit. As already mentioned, the nozzles **1601** are made of stainless steel but they can also be made of other conductive materials including copper, aluminum, gold and platinum. The nozzles **1601** are preferably coated with Teflon on both inner and outer surfaces. Each of the nozzles **1601** may typically have an outside diameter of 1.27 mm, an inside diameter of 0.8 mm and an overall length of 100 mm. With this size, each nozzle **1601** has an internal volume of ca. 60 μL which is sufficient for carrying out PCR and sequencing reactions. All reactions involved in PCR and sequencing are carried out within these nozzles **1601**. Hence, the nozzles **1601** serve as vessels in which to carry out the PCR and sequencing reactions.

The constituent material for the fixing plates **1621** is not limited to any particular type but they are preferably made of materials having both electrical insulating and heat resisting properties as exemplified by ceramics and plastics. They may of course be formed of metals. Similarly, the constituent material for the syringes **1615** is not limited to any particular type but they are preferably formed of materials having both electrical insulating and heat resisting properties as exemplified by glass and plastics. They may of course be formed of metals. The pistons **1617** are preferably made of glass or plastics. Alternatively, the syringes **1615** and the nozzles **1601** may be formed of the same metallic material (e.g. stainless steel) so that they can be rendered monolithic.

FIG. **5** is a schematic simplified view showing how to heat and cool the nozzles **1601**. The heating zone for the nozzles **1601** is defined by the two conductive boards **1609** which are spaced apart by a distance of 46 mm. The 96 nozzles are wired in series and connected between the two conductive boards **1609**. A dc voltage of 35.4 V (up to 43 V) is applied to produce a current of 8.2 A (up to 10 A). The heating time is 5 seconds (as calculated for heating up to 50° C.). These values assume that the nozzles used have an outside diameter of 1.27 mm and an inside diameter of 0.8 mm, are made of SUS **304** having and are heated over a distance of 46 mm. If other nozzle conditions are used, the voltage, current and heating time will have different values. The skilled artisan can readily determine the appropriate values of voltage, current and heating by repeated experiments. To secure appropriate temperature control, a temperature sensor such as a thermocouple (not shown) is preferably provided at suitable sites in the heating zone. As already mentioned, when the nozzles are heated, the shutters **1604** and **1607** are closed to shut off the nozzle heating space. In this case, air-cooled fan **1605** also stops operating. To cool the nozzles, voltage application is stopped and at the same time the shutters **1604** and **1607** are slid open and air-cooled fan **1605** is driven so that ambient air is blown against the outer surfaces of the nozzles.

The plates to be used in the apparatus of the invention for preparing DNA samples are common microtiter plates having a plurality of concavities or wells in the top surface. The plates may be formed of any suitable materials such as ceramics, glass and plastics. FIG. **6** is a partial simplified plan view of a microtiter plate **30** having 384 wells. Since every four wells are used to perform PCR and sequencing reactions, the plate **30** has 96 divisions consisting of 8 divisions a–h on the vertical axis and **12** divisions A–L on the horizontal axis. At coordinate Aa, there are four wells specified by **(1,1)**, **(1,2)**, **(2,1)** and **(2,2)** which are filled with the necessary reagents that are used at the respective stages of processing. For example, well **(1,1)** is filled with the reaction mixture and primers used in the first step of PCR; well **(2,1)** is used as a reaction well; well **(1,2)** is filled with a sequencing primer; and well **(2,2)** is filled with a sequencing reaction mixture. Amplification by PCR can be performed using a reaction mixture kit commercially available from Perkin-Elmer. The kit is a 30-μL solution consisting of 3 μL DNA template (e.g. ca. 200 ng human genome), 3 μL 10×PCR buffer, 3 μL (2.5 mmol) dNTP, 3 μL (10 μmol) primer **1**, 3 μL (10 μmol) primer **2**, 0.2 μL Ex Taq and 15 μL H_2O. Other reaction mixtures and primers can of course be used. The wells at the other coordinates Ab–Ah, Ba–Bh, Ca–Ch, . . . , La–Lh are filled with the same reagents as placed in the wells (1,1), (1,2), (2,1) and (2,2). Other reagents may of course be put in these wells. Depending on the use, the wells may be grouped in twos, threes, etc.

As FIG. **7** shows, in order to insert a nozzle **1601** into either one of the wells (1,1), (1,2), (2,1) and (2,2) that is determined by the specific step of processing, the plate **30** is moved in X-Y direction as it rests on the plate holding section **20**. Movement of the plate **30** in X-Y direction can be realized by adapting the plate holding section **20** to be movable in X-Y direction; the same result can be attained by allowing the arm of the transport robot **26** to hold the plate **30**. Alternatively, the nozzle **1601** itself may be moved in X-Y direction.

FIG. **8** is a simplified perspective view of an exemplary structure of the enzyme supply section **18** where the enzymes used in PCR and which remain unreacted are decomposed and purified. In the process of sample preparation, a centrifuge is conventionally used to purify the PCR amplified products. The apparatus of the invention allows for full automation of PCR and sequencing reactions by using enzymes. Two enzymes are used; one is alkaline phosphatase shrimp (APS) for removing unreacted dNTP and the other is exonuclease I (Exol) for removing unreacted primers. Other decomposing enzymes may of course be used. Each of these enzymes is diluted 20-fold with, for example, TE and supplied in portions of a predetermined quantity, say, 2 μL. The enzyme supply section **18** comprises basically a rectangular container **1803** with its top open and a closed enzyme tank **1805** coupled monolithically to it. The container **1803** has a plurality of, say, 96, enzyme supply pots **1801** erected on the bottom. An enzyme solution is supplied through an inlet **1809** that is formed in the top surface of the enzyme tank **1805** and which is fitted with an openable lid **1807**. The enzyme solution can be supplied by any suitable means that is not shown in FIG. **8** or it may be supplied manually by operating personnel. The enzyme tank **1805** is preferably provided with a level sensor **1811** for detecting how much of the enzyme solution is left in the tank **1805**. The supply of the enzyme solution into the tank **1805** is controlled in response to a detection signal from the level sensor **1811**.

The enzyme supply section **18** also has a solution transfer mechanism **1813** for transferring the enzyme solution from the enzyme tank **1805** into the enzyme supply pots **1801**. The solution transfer mechanism **1813** typically uses a stepping motor **1815** which rotates a precision feed screw **1817** so that a piston **1821** is moved back and forth through a syringe **1819**. The precision feed screw **1817** and the piston **1821** are coupled by a connecting arm **1823**.

Decomposition enzymes such as alkaline phosphatase shrimp (APS) and endonuclease I (Exol) are occasionally deactivated at elevated temperatures. To deal with this possibility, the bottoms of the rectangular container **1803** and the enzyme tank **1805** are entirely covered with a cooling unit **1825**. Preferably, the cooling unit **1825** maintains the enzyme solution at about 4° C. which is low enough to keep the enzymes dormant.

FIG. **9** is a partial enlarged section of the solution at transfer mechanism **1813** shown in FIG. **8**. A generally T-shaped pipe **1827** is connected to the distal end of the syringe **1819**. A check valve **1829** is connected to an end of the T pipe **1827** and another check valve **1831** is connected to the other end of the T pipe. The other end of the check valve **1829** is connected to a pipe **1833** from the enzyme tank **1805**; the other end of the check valve **1831** is connected to a pipe **1835** branching into the enzyme supply pots

1801. If the piston **1821** is pulled, the enzyme solution from the enzyme tank **1805** passes through the check valve **1829** to fill the syringe **1819**. On this occasion, the check valve **1831** remains closed, so the enzyme solution will not flow into the enzyme supply pots **1801**. If the piston **1821** is pushed in, the enzyme solution in the syringe **1819** passes through the check valve **1831** to be transferred into the enzyme supply pots **1801**. On this occasion, the check valve **1829** remains closed, so the enzyme solution in the syringe **1891** will not return to the enzyme tank **1805**.

FIG. **10** is a partial enlarged section of enzyme supply pots **1801**. The pipe **1835** branches in supply pipes **1837** which are connected to the bottoms of the enzyme supply pots **1801**. If the piston **1821** is pushed in, the enzyme solution in the syringe **1819** passes through the check valve **1831** and flows through the pipe **1835** and branch pipes **1837** until it fills up the pots **1801** from bottom to top. If a nozzle **1601** is inserted into the pot **1801**, the enzyme solution in the pot **1801** may occasionally overflow; the overflowing enzyme solution falls into an emission space **1839** adjacent each pot **1801** and thereafter flows through a liquid waste drain **1841** to be discarded to the outside of the container **1803**.

FIG. **11** is a simplified sectional view showing an exemplary structure of the nozzle sealing section **22**. As shown, the nozzle sealing section **22** comprises a tray **2203** filled with paraffin **2201**, a heating/cooling means **2205** bonded to the underside of the tray **2203**, and a heat sink **2207** bonded to the other side of the heating/cooling means **2205**. It should be noted that the heat sink **2207** is not essential to the present invention. The heating/cooling means **2205** is typically a Peltier device.

If thermal cycles are applied to the sample reaction liquor in a conductive nozzle **1601** (see FIG. **4**) so as to perform PCR and sequencing reactions in the nozzle, air bubbles within the nozzle can expand at elevated temperatures and the sample reaction solution may be ejected out of the nozzle. In order to avoid this problem, the tip of the conductive nozzle **1601** is sealed with paraffin **2201** before thermal cycling starts. This paraffin sealing has enabled all PCR and sequencing reactions to be performed in a fully automatic manner.

FIG. **12** shows schematically the sequence of steps in paraffin sealing. In step A, the Peltier device **2205** is driven to heat the tray **2203** so that the paraffin in the tray **2203** is melted. The piston **1617** is lifted up only a little to form an empty space at the tip of the nozzle **1601** which is filled with the sample solution. In step B, the nozzle **1601** is lowered down so that its tip is immersed in the molten paraffin, whereupon the paraffin **2201** gets into the empty space at the tip of the nozzle **1601**. In step C, the Peltier device **2205** is driven in reverse direction to cool the tray **2203** so that the paraffin in the tray **2203** is solidified, whereupon the tip of the nozzle **1601** is completely sealed with the paraffin. With the paraffin bath **2203** being cooled with the Peltier device **2205** (to keep the paraffin **2201** solidified), an electric current is flowed through the nozzle **1601** as shown in FIG. **5** (with its tip paraffin-sealed) so that it is heated to the temperature necessary for performing PCR or sequencing reactions and a predetermined reaction is carried out within the nozzle; thereafter, the application of the current is stopped, the nozzle **1601** is cooled and a predetermined thermal cycle is repeated. Since the tip of the nozzle **1601** is not heated, the paraffin seal is not melted but remains solidified. If the predetermined thermal cycle is repeated to complete the necessary PCR or sequencing reactions, the process goes to step D, in which the Peltier device **2205** is driven to heat the tray **2203** so that the paraffin in the tray is melted. If the paraffin **2201** is completely melted, the nozzle **1601** is lifted up from the tray (paraffin bath). Since the paraffin sealing the tip of the nozzle **1601** is also melted, the tip of the lifted nozzle **1601** returns to the initial emptiness (see step A). As already mentioned, the nozzle **1601** is coated with Teflon on both the inner and outer surfaces, the liquid paraffin can be readily removed from the tip of the nozzle **1601**.

Alternatively, the tip of the nozzle **1601** may be pressed onto the surface of a mat which is indicated by **2210** in FIG. **13** and this produces the same result as the paraffin sealing strategy illustrated in FIGS. **11** and **12**. To be more specific, the tip of the nozzle **1601** which is not filled with the sample solution is pressed onto the mat **2210** and with it remaining in this state, an electric current is flowed through the nozzle **1601** so that it is heated to the temperature necessary for performing PCR or sequencing reactions and a predetermined reaction is carried out within the nozzle; thereafter, the application of the current is stopped, the nozzle **1601** is cooled and a predetermined thermal cycle is repeated. Since the empty space at the tip of the nozzle **1601** has the necessary and sufficient capacity, the sample will not be ejected out of the nozzle even if it is heated to expand. If the predetermined thermal cycle is repeated to complete the necessary PCR or sequencing reactions, the nozzle **1601** is lifted up from the surface of the mat **2210** and moved to the next step of processing. In FIG. **13**, reference numeral **2212** designates the support of the mat **2210**.

If the tip of a different nozzle **1601** is pressed onto the surface of the mat **2210** at the same site as where the previous nozzle was pressed to seal its tip, contamination might occur. To avoid this risk, the tip of a nozzle **1601** for performing a second cycle of PCR amplification must always be pressed onto the mat **2210** at a site that has not been used before. To meet this requirement, the mat **2210** is preferably moved back and forth and/or to right and left by a known conventional means (not shown). The mat may be sheet fed so that it is replaced by a new one at each end of the entire process of PCR amplification. If desired, a web of continuous sheet may be delivered little by little for each process of PCR amplification. In FIG. **14**, reference numeral **2214** designates a tension roll, **2216** is a delivery roll, and **2218** is a take-up roll. By using the device shown in FIG. **14**, the position of the mat is changed at each end of the process of PCR amplification and there is no possibility that the PCR amplified products will contaminate the next process of PCR amplification. As a result, the apparatus of the invention for preparing DNA samples can be run continuously for extended periods.

Being used for the stated purpose, the mat **2210** can be formed of materials that are heat resistant, flexible, elastic and non-conductive. Preferred examples of such materials include fluoroplastics, fluororubbers, urethane rubber, silicone rubber and chloroprene rubber. It is generally preferred that the mat has a thickness in the range of 1.0–10 mm. If the mat is thinner than 1.0 mm, the tip of the nozzle cannot be adequately sealed and, in addition, the mat may be broken by the tip of the nozzle. If the mat is thicker than 10 mm, the effect of sealing the tip of the nozzle is saturated and diseconomy results; in addition, such a thick mat is difficult to handle. Particularly in the case of using the web of continuous sheet shown in FIG. **14**, the mat thickness is preferably in the range of 1.0–5.0 mm.

FIG. **15** is a partial simplified sectional view showing an exemplary structure of the cleaning tank section **24**. As shown, the cleaning tank section **24** consists of a cleaning tank **2401** which contains in it a plurality of cleaning pots **2403** of a specified height and a plurality of drain trenches **2405**. The cleaning tank **2401** may have a rectangular shape. The bottom of each cleaning pot **2403** communicates with a cleaning water inlet **2407**. The cleaning water may be selected from various kinds of impurity-free water such as pure water, deionized water and distilled water. A suitable pipe or the like may be provided between a supply source of cleaning water (not shown) and each of the cleaning water inlets **2407**. The capacity of each cleaning pot **2403** is preferably the same as or slightly greater than the internal volume of the nozzle. During the cleaning process, the cleaning pots **2403** are preferably kept supplied with fresh cleaning water so that it keeps overflowing these cleaning pots. While the fresh cleaning water keeps overflowing the cleaning pots **2403**, the tip of a nozzle **1601** is immersed into the cleaning water from above the cleaning pot **2403**. When the piston **1617** is advanced into the cleaning pot **2403**, the cleaning water is discharged from the nozzle **1601** and by retracting the piston **1617**, the cleaning water is sucked into the nozzle **1601**; in this way, the interior of each nozzle **1601** can be cleaned. The cleaning water may be discharged into the cleaning pot **2403** or the drain trench **2405**. Preferably, the cleaning water is discharged into the drain trench **2405** in order to keep the interior of the cleaning pot **2403** clean. The cleaning water may be sucked in and discharged in one or more cycles. The cleaning water discharged from the nozzles **1601** and the cleaning water overflowing the cleaning pots **2403** flow through a drain hole **2409** in the bottom of the drain trench **2405** to collect in a suitable drain tank (not shown) provided outside the cleaning tank **2401**. Cleaning of the nozzles **1601** is not the sole function of the cleaning tank **2401** and it can also be used for other purposes such as diluting the reaction products with pure water.

EXAMPLE

FIG. **16** is a flowchart for the sequence of steps in a process by which DNA samples labelled with a single fluorescent dye are prepared with the apparatus of the invention for preparing DNA samples. The process gets started in step **1401**. At this stage, the apparatus of the invention for preparing DNA samples is fully prepared for the making of DNA samples by being equipped with all necessary materials and devices such as enzyme solutions, cleaning water and microtiter plates. Thus, the transport robot **26** picks up a supply plate **30** from the plate stacker **28** and places it in the plate holding section **20**. In step **1402**, the reactor **16** is moved to the position of the plate holding section **20** and lowered down so that a nozzle **1601** sucks 20 μL out of 24 μL of the reaction mixture plus primers that are within the well in the plate **30** which is located at coordinate (1,1). In subsequent step **1403**, the reactor **16** is moved to the nozzle sealing section **22** and lowered down to seal the tip of the nozzle with paraffin and carry out PCR; after predetermined thermal cycles are completed, the paraffin seal is removed. In step **1404**, the nozzle is moved to the empty well in the plate which is located at coordinate (1,2) and all contents of the nozzle are dispensed into this empty well. In step **1405**, the nozzle sucks in 5 μL of purifying decomposition enzymes and adds them to the contents of the well provided in step **1404**. The nozzle sucks in 20 μL of the resulting mixture. Subsequently, in step **1406**, the nozzle tip is sealed with paraffin and the nozzle is heated so that the unreacted residues (dNTP and primers) in the mixture are purified by enzymatic decomposition. In step **1407**, 3 μL of the purified product is dispensed into a cleaning pot **2403** and the rest discarded. Thereafter, 60 μL of pure water is forced into the cleaning pot to dilute the purified product. In step **1408**, 1 μL of the dilution is sucked into the nozzle and added to the primers in the well at coordinate (2,1) to make a total of 8.3 μL. In step **1409**, 7 μL of the primer mixture in the well (2,1) is sucked into the nozzle and all added to 5 μL of the reaction mixture in the well (2,2); the resulting mixture is fully agitated. In step **1410**, 12 μL of the resulting mixture is sucked into the nozzle, whose tip is sealed with paraffin for sequencing reactions. After the end of sequencing reactions, the paraffin seal is removed. In step **1411**, 12 μL of the mixture is dispensed into each well in a fresh 96-well plate. In step **1412**, determination is made as to whether the sequencing reaction product should be purified. If the answer is negative, the sequence goes to step **1413** and the transport robot **26** replaces the 96-well plate in a predetermined tray in the plate stacker **28**, whereupon the sequence of the necessary steps ends. If purification is necessary, the sequence goes to step **1414** and the necessary purification is performed not with the apparatus of the invention but with Model SG-8GC of PSS. The purified product is obtained in step **1415**. The purified product is sucked into the nozzle and in step **1416** it is dispensed into a predetermined well in a recovery sample plate. Thereafter, the sequence goes to step **1417** and the transport robot **26** replaces the recovery sample plate in a predetermined tray in the plate stacker **28**, whereupon the sequence of the necessary steps ends.

In the flowchart shown in FIG. **16**, a single fluorescent dye is used to label DNA samples but this is not the sole case of the invention and more than one fluorescent dye may be used to label DNA samples. The process is the same except on the following points: the total volume prepared in step **1408** is increased to 16 μL; in step **1409**, 4 μL of the reaction mixture is added to make a total of 20 μL which is used in sequencing reactions in step **1410**.

FIG. **17** is a flowchart for performing purification (see step **1414** in FIG. **16**) by Model SG-8GC of PSS. The process starts with step **1501** using a sample plate containing the liquid products of sequencing reactions. This sample plate is the same as the 96-well plate used in step **1411** in FIG. **16**. In step **1502**, a beads fluid is added and the resulting mixture is sucked in and dispensed in 20 μL. The dispensed mixture (20 μL) is stirred several times in step **1503**, incubated at room temperature for 5 minutes in step **1504**, given magnetism and left standing for 1 minute in step **1505**. In step **1506**, 70% ethyl alcohol is added and the resulting mixture is sucked in and dispensed in 50 μL. The dispensed mixture is stirred 10 times in step **1507**. In step **1508**, magnetism is applied again and the mixture is left standing for 1 minute. In step **1509**, the supernate is removed. In step **1510**, 70% ethyl alcohol is added again and the resulting mixture is sucked in and dispensed in 50 μL. In step **1511**, the dispensed mixture is stirred 10 times. In step **1512**, magnetism is applied again and the mixture is left standing for 1 minute. In step **1513**, the supernate is removed. The subsequent process depends on whether the purified product obtained in step **1513** is subjected to gel electrophoresis or capillary electrophoresis. If it is to be subjected to gel electrophoresis, the sequence goes to step **1514**, in which a buffer solution is added to the purified product and the resulting mixture is sucked in and dispensed in ca. 2–5 μL in a fresh 96-well plate. The purification process ends in step **1515**. If the purified product is to be subjected to capillary electrophoresis, the sequence goes to step **1516**, in which a buffer solution is added to the purified product and the resulting mixture is sucked in and dispensed in ca. 10–20 μL. In step **1517**, all contents of the well are sucked in and heated at 80–90° C. for 2 minutes. In step **1518**, the supernate is recovered. The purification process ends in step **1519**.

As will be apparent from the foregoing description, the present invention provides an integrated system by which PCR and sequencing reactions can be performed with a single unit of apparatus and this allows for fully automated preparation of DNA samples for use in DNA sequencing. As a result, the throughput of DNA sample preparation is significantly improved.

What is claimed is:

1. An apparatus for automated preparation of DNA samples which comprises a reactor for preparing DNA samples and adjacent thereto an enzyme supply section, a plate holding section, a nozzle sealing section and a cleaning tank section, and wherein plates are loaded onto or unloaded from said plate holding section by means of a transport robot.

2. The apparatus according to claim **1**, wherein said reactor for preparing DNA samples is supported on a unidirectionally moving mechanism to be capable of moving up and down and comprises a plurality of hollow electroconductive nozzles, hollow syringes coupled to said nozzles and pistons inserted into said syringes, the top of each of said pistons having a piston head secured thereto such that it can move up and down independently of said reactor for preparing DNA samples, the intermediate portions of said electroconductive nozzles being encased in a housing having an opening on both sides, a cooling mechanism being provided adjacent one of said openings, and electroconductive boards being connected to the intermediate portions of the electroconductive nozzles within said housing and also connected to a power supply via conductors.

3. The apparatus according to claim **2**, wherein said nozzles are formed of an electroconductive metal material selected from the group consisting of stainless steel, copper, aluminum, gold and platinum, and said nozzles are coated with Teflon on both inner and outer surfaces.

4. The apparatus according to claim **2**, wherein said nozzles are used as reaction vessel for PCR and sequencing reactions.

5. The apparatus according to claim **4**, wherein each of said nozzles has an outside diameter of 1.27 mm, an inside diameter of 0.8 mm, an overall length of 100 mm and an internal volume of ca. 60 μL.

6. The apparatus according to claim **2**, wherein said electroconductive boards are spaced apart by a distance of 46 mm and said nozzles are 96 in number.

7. The apparatus according to claim **2**, wherein said cooling mechanism is a cooling fan and which further includes at least one straightening plate between said two electroconductive boards for straightening the air flows created by said cooling fan.

8. The apparatus according to claim **7**, wherein openable shutters are provided at the openings on both sides of said housing and said cooling fan is provided adjacent one of said shutters.

9. The apparatus according to claim **1**, wherein said enzyme supply section comprises a rectangular container with an open top having a plurality of enzyme supply pots erected in the interior, a closed enzyme tank coupled monolithically to said rectangular container, a solution transfer mechanism for transferring an enzyme solution from said enzyme tank to said enzyme supply pots, and a cooling means provided on the underside of said rectangular container.

10. The apparatus according to claim **1**, wherein said nozzle sealing section has at least a paraffin-filled tray and a heating/cooling means provided on the underside of said tray.

11. The apparatus according to claim **10**, wherein said heating/cooling means is a Peltier device and which further includes a heat sink bonded to the side of said Peltier device which is remote from the side bonded to said tray.

12. The apparatus according to claim **1**, wherein said nozzle sealing section comprises a mat formed of a heat-resistant, flexible, elastic and non-conductive material and said nozzle is sealed by pressing its tip onto the surface of said mat.

13. The apparatus according to claim **12**, wherein said mat is formed of a material selected from the group consisting of fluoroplastics, fluororubbers, urethane rubber, silicone rubber and chloroprene rubber.

14. The apparatus according to claim **12**, wherein said mat is either sheet-fed or a web of continuous sheet.

15. The apparatus according to claim **1**, wherein said cleaning tank section comprises a cleaning tank which is a rectangular container with an open top having a plurality of cleaning pots erected in the interior and which has drain trenches adjacent said cleaning pots, said cleaning pots being supplied with cleaning water from the bottom, and said drain trenches communicating with a drain hole.

16. A reactor for preparing DNA samples which comprises a plurality of hollow electroconductive nozzles, hollow syringes coupled to said nozzles and pistons inserted into said syringes, the top of each of said pistons having a piston head secured thereto such that it can move up and down independently of said reactor for preparing DNA samples, the intermediate portions of said electroconductive nozzles being encased in a housing having an opening on both sides, a cooling mechanism being provided adjacent one of said openings, and electroconductive boards being connected to the intermediate portions of the electroconductive nozzles within said housing and also connected to a power supply via conductors.

17. The reactor according to claim **16**, which is supported on a unidirectionally moving mechanism to be capable of moving up and down and wherein said piston head can move up and down independently of said reactor.

18. The reactor according to claim **16**, wherein said nozzles are formed of an electroconductive metal material selected from the group consisting of stainless steel, copper, aluminum, gold and platinum, and said nozzles are coated with Teflon on both inner and outer surfaces.

19. The reactor according to claim **16**, wherein said nozzles are used as reaction vessel for PCR and sequencing reactions.

20. The apparatus according to claim **19**, wherein each of said nozzles has an outside diameter of 1.27 mm, an inside diameter of 0.8 mm, an overall length of 100 mm and an internal volume of ca. 60 μL.

21. The reactor according to claim **16**, wherein said electroconductive boards are spaced apart by a distance of 46 mm and said nozzles are 96 in number.

22. The reactor according to claim **16**, wherein said cooling mechanism is a cooling fan and which further includes at least one straightening plate between said two electroconductive boards for straightening the air flows created by said cooling fan.

23. The reactor according to claim **22**, wherein openable shutters are provided at the openings on both sides of said housing and said cooling fan is provided adjacent one of said shutters.

24. The reactor according to claim **16**, which is used together with an enzyme supply unit, a nozzle sealing unit and a cleaning unit.

25. The reactor according to claim **24**, wherein said enzyme supply section comprises a rectangular container with an open top having a plurality of enzyme supply pots erected in the interior, a closed enzyme tank coupled monolithically to said rectangular container, a solution transfer mechanism for transferring an enzyme solution from said enzyme tank to said enzyme supply pots, and a cooling means provided on the underside of said rectangular container.

26. The reactor according to claim **24**, wherein said nozzle sealing section has at least a paraffin-filled tray and a heating/cooling means provided on the underside of said tray.

27. The reactor according to claim **26**, wherein said heating/cooling means is a Peltier device and which further includes a heat sink bonded to the side of said Peltier device which is remote from the side bonded to said tray.

28. The reactor according to claim **24**, wherein said nozzle sealing section comprises a mat formed of a heat-resistant, flexible, elastic and non-conductive material and said nozzle is sealed by pressing its tip onto the surface of said mat.

29. The reactor according to claim **28**, wherein said mat is formed of a material selected from the group consisting of fluoroplastics, fluororubbers, urethane rubber, silicone rubber and chloroprene rubber.

30. The reactor according to claim **28**, wherein said mat is either sheet-fed or a web of continuous sheet.

31. The reactor according to claim **24**, wherein said cleaning tank section comprises a cleaning tank which is a rectangular container with an open top having a plurality of cleaning pots erected in the interior and which has drain trenches adjacent said cleaning pots, said cleaning pots being supplied with cleaning water from the bottom, and said drain trenches communicating with a drain hole.

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